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Preplanting Treatment for Billbug Control on Corn[1]

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In many areas of the United States the major cause of loss of stand in field corn is the feeding of one or more species of billbugs. The most prevalent species in South Carolina is the maize billbug, *Calendra maidis* (Chitt.) (Blatchley & Leng 1916). With minor variations, the life history of this species and types of injury it causes are similar to those of most other species of billbugs attacking corn.

These insects may start to feed on seedling corn as soon as the plant emerges from the soil, and continue feeding until after the corn is well over 6 feet high and aerial brace roots have formed. In fields heavily infested stands can be completely destroyed even through the fourth planting, after which it is too late in the season to replant. Feeding occurs at or below the ground level. The first indication of injury is the wilting of the center leaves or "bud" resulting from the adult billbug feeding on the rolled-up base of these leaves. When plants under 3 inches high are attacked, they usually wilt and die. Larger plants occasionally outgrow the injury, provided the growing point is not injured; otherwise, suckers develop and the plant is nonproductive.

Several other insects cause injury to corn which superficially resembles that caused by billbugs, but positive diagnosis can be made either by finding the adult at the base of the plant or by finding the narrow feeding slit in the base of the outer leaves where they are wrapped around to form the stem of the seedling. This slit is generally about $\frac{1}{32}$-inch wide and $\frac{1}{4}$- to $\frac{1}{2}$-inch long, running lengthwise of the stem. In somewhat older stands of corn, stunted or misshapen plants may also have the off-white, legless, brownheaded larval stages present inside the stalk at about the ground level.

Control of billbugs attacking corn is particularly difficult, for the insect is found within the corn stalk from the time the egg is laid in the stalk until the adult emerges from the stalk in the spring to mate and search for food. When the adult finds corn to feed on, it usually crawls down the base of the plant until it is partly or completely below the ground level. Contact sprays or dusts are impractical for control of insects thus hidden. Residual materials applied to the surface of the soil are degraded by the effects of sunlight, and therefore are effective for only a short period. As billbugs emerge from hibernation over a 2- to 4-week period, several applications of a residual insecticide would be required to maintain sufficient residue on the soil surface to give control. Surface applications would also have to be repeated after each cultivation. While this might be practical for some crops, the low per-acre value of corn makes repeated applications impractical.

Fortunately, control measures are somewhat simplified by the inability of most billbugs to fly. This enables the grower to predict rather accurately where an infestation will cause damage, once he knows it is present. This information is obtained by a fall survey of corn fields as previously described by Kirk (1956).

Previous work showed control by cultural methods such as rotation, early planting, use of adequate amounts of starter fertilizer, or replanting, considerably more effective than any chemical controls tried. Most of these chemical controls consisted of surface-row applications applied after the corn was up and the billbugs had started to feed. Billbugs were killed by some of the materials, but the kill obtained was seldom enough to save the stand.

Insectary and field cage tests previously reported by Kirk (1956) showed that various methods of surface applications of insecticides failed to save the stand from billbug injury, but that a broadcast, disked-in, preplanting application indicated considerable promise. Of the residual materials tested by this latter method, aldrin at 2 pounds of actual toxicant per acre resulted in the highest mortality. Three heavily infested fields receiving this treatment had virtually no injury from billbugs, whereas two similar untreated check fields had 51 and 62 %, respectively, of the stand destroyed.

Following these promising results, a 2-year large-scale field testing program was set up to corroborate these results prior to formulating a control recommendation for use by the growers.

1956. FIELD TEST.—Thirteen fields with a total of 59 acres of cornland were found by fall survey to have heavy infestations of billbugs. Corn in these fields was found to have an average of 62% of the stalks infested in the fall of 1955, and no field had less than 50% infestation. Based on previous observations, this would have resulted in virtually a complete loss of stand in 1956, had these fields been planted to corn and not treated. These fields were treated between March 21 and April 3, 1956, with a broadcast, disked-in, preplanting application of 5 pounds per acre of 40% aldrin wettable powder mixed with 95 pounds of fertilizer as a diluent. Whole fields, from 0.5 to 10 acres each, were treated to control the entire infesta-

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Table 1.—Results of broadcast, disked-in, preplanting application of 2 pounds of actual aldrin per acre for control of billbugs on corn, Florence, S. C. 1956.

Field No.	Acres	Per Cent Fall Infestation	Per Cent Plants Injured Following Treatment
Treated			
1	5	54	3.6
2	3	80	4.4
3	1	68	4.5
4	3	60	4.5
5	9	50	4.5
6	3	50	7.5
7	12	60	15.2
8	10	60	19.1
9	1	66	24.3
10	2	55	24.9
11	6	90	25.7
12	2	50	37.7
13	2	70	40.3
			Average 16.7
Check			
1	4	68	96.1
2	2	52	83.0
3	5	60	91.5
			Average 90.2

tion in each case. Three similar fields were left untreated as checks. The results are summarized in table 1.

Loss of stand from billbugs following this treatment ranged from 3.6% in one field to 40.3% in another, with an over-all average of 16.7% injured plants for the 59 acres treated. The untreated check fields with similar overwintering infestations had 83.0 to 96.1% loss of stand, with an average of 90.2%.

Insectary Tests.—Results reported in 1956 (Kirk) showed virtual eradication of billbugs in treated fields, therefore the control obtained in the 1956 field test, though much better than any control previously obtained by chemicals, was not as good as anticipated. As soil composition might have been the major variant causing this wide range of effectiveness, insectary tests were conducted to determine the influence of this factor on the efficiency of aldrin. Experiments were designed to determine whether the three major soils in the Coastal Plains area of South Carolina required different amounts of aldrin to give equal billbug control. These soils are loamy sand, loamy clay, and sandy loam high in organic matter, and are commonly referred to as sandy soil, clay, and bottom land.

Aldrin wettable powder was thoroughly mixed with air-dried samples of each of the above soils to give the equivalent of 0, 2, 4, 8, and 16 pounds of actual insecticide per acre. The following rates of water were then added to each 3 pound sample: sandy soil and clay—150 cc., bottom land—175 cc. Samples were then divided into three replicates in 4-quart cans, 10 billbugs added to each, a glass cover put on, and placed in a constant temperature room at 80° F. for 4 days. The results are summarized in table 2.

Aldrin at 2 pounds per acre resulted in 100% control in both sandy soil and clay, but 16 pounds per acre were required to give equivalent control with the soil from bottom land, under the conditions of these tests. Under field conditions of longer exposure, however, it is possible that 8 pounds, or possibility even 4 pounds of actual aldrin per acre would result in good or excellent control.

Since 2 pounds of aldrin in clay soil resulted in as good control as 2 pounds in sandy soil, it appears that the colloidal nature of the soil did not tie up appreciable amounts of aldrin. The organic component of the soil from the bottom land apparently lowered the effectiveness of the insecticide in those samples.

1957. Field Tests.—Ten fields with a total of 39 acres of cornland having heavy billbug infestations were treated with aldrin between March 21 and April 30. As in 1956, complete fields were treated, except two that were bottom land. In accordance with the results shown in table 2, these two fields received 4 pounds of actual aldrin per acre. This rate was chosen because it represents the maxi-

Table 2.—Effect of soil type on effectiveness of aldrin for billbug control. Florence, S. C. 1957.

Soil Type	Pounds Aldrin per Acre	Per Cent Dead and Moribund Billbugs					
		Test Number					
		1	2	3	4	5	Average
Loamy Sand	2	100	100	100			100
	4	100	100	100			100
Loamy Clay	2	100	100	100			100
	4	100	100	100			100
Sandy Loam[a]	2	26.6	33.3	13.3	20.0	20.0	22.6
	4	46.6	56.6	56.6	53.3	43.3	51.3
	8			90.0	73.3	76.6	79.9
	16				100.0	100.0	100.0
Checks (average)		0.0	0.0	2.5	0.0	0.0	0.5

[a] Bottom land.

Table 3.—Results of broadcast, disked-in, preplanting application of aldrin for control of billbugs on corn. Florence, S. C. 1957.

Field No.[a]	Acres	Pounds Actual Aldrin per Acre	Per Cent Fall Infestation	Per Cent Plants Injured Following Treatment
Treated				
1	9	2	75	1.00
2	2	2	30	.25
3	3	2	30	.50
4	4	2	50	.25
5	3	2	55	.50
6	2	2	20	.50
7	3	2	50	.50
8	4	2	80	.20
				Average 0.46
9	2	4	60	1.00
10	4	4	85	.10
				Average 0.55
Check	1	—	50	70.00
11	4	—	30	80.00
12	2	—	85	42.00
13	6	—	50	34.00
				Average 56.50

[a] Fields 1 to 8 were light soil; fields 9 and 10 were heavy soil or bottom land.

mum amount growers could afford to pay to obtain control of billbugs. Four infested fields were left untreated as checks. The results are summarized in table 3.

The 1956 fall survey showed infestations in the fields ranged from 20 to 85% of the stalks infested, with an average of 53%.

Such excellent control of billbugs resulted from the above treatment that injury was reduced to from 1 to 0.1% in all treated fields, with an over-all average of ½ per cent of the plants injured. The 4-pound rate used on the bottom-land fields was as effective as the 2-pound rate used on the other soils. The four untreated check fields with similar over-wintering infestations had 34 to 80% of the plants injured, with an average of 56.5%.

Previous observations (Kirk, 1956) indicated that if corn were to be planted in a field adjacent to an infested field, the infested field should be treated, rather than the one being planted to corn. In an attempt to corroborate these observations, two fields in which heavy infestations of billbugs were found during the fall survey were subsequently planted in oats. These oats were not disturbed the following spring, but the adjacent fields were treated with aldrin prior to planting corn. This treatment resulted in virtually no control for about 200 feet into the new corn, and only slight control further into the field, thus confirming the previous observations.

SUMMARY.—A 2-year program was conducted to corroborate previously reported data which indicated a broadcast, disked-in, preplanting application of aldrin resulted in excellent control of billbugs (*Calendra maidis* (Chitt.)) on corn. In 1956, damage following treatment ranged from 3.6% to 40.3%. Subsequent insectary studies showed that the more organic soils required higher rates of insecticide to give control equivalent to 2 pounds aldrin on the more mineral soils. In 1957, 2 pounds aldrin per acre on mineral soils and 4 pounds on organic soil resulted in reduction of damage to from 1 to 0.1%. The untreated fields averaged 56.5% loss of stand.

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Experiments with Several Repellent Formulations Applied to Cattle for the Control of Stable Flies[1]

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In the past several years numerous chemicals have been screened in laboratory tests to find a few exhibiting sufficient repellency to stable flies (*Stomoxys calcitrans* (L.)) to justify more extensive field evaluations.

Needless to say, much confusion has arisen from published reports on tests involving mixtures of repellents and toxicants and mixed populations of stable flies and horn flies (*Siphona irritans* (L)). In an average field population of biting flies one can expect 80 to 90% of the flies to be horn flies. Consequently, when a repellent with a killing agent such as methoxychlor or activated pyrethrins is applied to the cattle, one can expect the insecticidal residue to continue to kill horn flies for a period of 1 to 3 weeks, depending upon the dosage of the toxicant. If the results of such experiments are reported as repellency to horn flies and stable flies, the data are unreliable, inasmuch as one does not know the actual degree of repellency attained for either species. Actually such data may represent only the killing action of the toxicant on the horn fly and no repellency whatsoever to the stable fly. This may have been a factor contributing to the results obtained by Granett & Haynes (1955) and Granett & Hansens (1956).

METHOD of STUDY.—The experiments reported here have been confined to the repellent action of chemicals against stable flies, since the stable fly population persists even in the presence of the toxicants used in the repellent formulations. A conventional method of selecting animals on the basis of fly susceptibility by pretreatment fly counts (Fryer *et al.* 1948) was used in all tests. Each repellent was applied to three cows of approximately equal fly susceptibility. The number of replications was varied according to the precision desired in the data obtained and to some extent according to the time available for conducting the experiments. Stable fly counts were made at times when flies are generally most active. Usually the observers made three or more counts of actively feeding stable flies on each visit to a herd in order to increase the reliability of the data for that particular visit. Percentage repellency was based upon average number of flies found upon treated and untreated animals and corrected in the usual way for differences in animal group susceptibility. The methods of application, dosage, and pertinent observations are given with each of the tests.

OIL BASE SPRAY APPLICATIONS.—During 1955 a number of spray formulations were compared for their relative effectiveness in repelling stable flies. In the first of these tests low dosages (20 ml. to each cow) were applied to three cow groups at 5:00 to 5:30 A.M. by means of a small hand-operated atomizing sprayer. The small amount of spray applied to each cow was expected to have a rather short-lived repellency to the stable fly and thereby reveal the relative repellency during the first day of observation.

Results shown in table 1 are from eight replicates and indicate percentage repellency for the different formula-

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Table 1.—Per cent repellency of various oil solution formulations to stable flies through 12 hours.[a]

Formulation[b]	Per Cent Repellency			
	Hours After Treatment			
	4	6	8	12
AT4	79.4	71.8	53.0	38.6
AT5	77.0	65.3	45.0	32.4
AT6	77.0	71.8	64.5	16.2
5% R-326	83.7	69.5	57.5	51.7
5% R-326				
50% BPG[c]	75.6	61.5	57.2	38.2
2% R-326				
10% MGK 264	78.9	76.7	64.9	62.2
2% R-326	88.5	60.0	47.8	47.8
L.S.D. at 5% level	8.14	15.672	26.72	20.60
at 1% level	10.97	36.79	36.02	27.78
Average number of stable flies on untreated cows				
	20.9	26.2	28.7	30.4

[a] The formulations were applied to groups of three cows for eight replications at a dosage of 20 ml. per cow.
[b] AT4, AT5, and AT6 were made up of 50%, 40%, and 30% of butoxy polypropylene glycol, respectively, plus 2% piperonyl butoxide plus 0.2% pyrethrins.
[c] Butoxy polypropylene glycol.

tions. Little or no correlation between percentage of butoxy polypropylene glycol (Crag Fly Repellent) and percentage reduction in stable flies is evident in formulations AT4, AT5, and AT6, which contained 50%, 40%, and 30% butoxy polypropylene glycol, respectively. Inas much as the relatively high level of pyrethrin (0.2%) plus piperonyl butoxide (2%) is in itself an effective repellent, a question naturally arises as to whether or not the presence of the pyrethrins masked the repellent action of the butoxy polypropylene glycol in the formulations tested.

R-326 (di-N-propyl isocinchomeronate) proved to be an effective stable fly repellent in the four formulations tested. The repellent properties of R-326 were enhanced by the addition of MGK 264, but unaffected by the addition of butoxy polypropylene glycol, table 1.

In the second experiment, table 2, 2% R-326 in mineral seal oil was compared with a spray concentrate containing 10% piperonyl butoxide and 1% pyrethrins. In three replicates of three animals each, spray was applied at a dosage of 60 ml. per cow. A high level of repellency was exhibited by both formulations. Warm, dry weather conditions prevailed during these tests, which may be responsible for the relatively high performance values of R-326.

Table 2.—Per cent repellency of oil based sprays to stable flies through 12 hours.[a]

Formulation	Per Cent Repellency			
	Hours After Treatment			
	4	8	10	12
2% R-326 in base oil	100.0	99.5	71.2	81.4
10% piperonyl butoxide & 1% pyrethrins	97.7	100.0	76.4	74.3
Average number of stable flies on untreated animal				
	9.8	21.1	18.9	28.1

[a] Groups of three treated cows were sprayed with 60 ml. per cow at 6:00 A.M. No significant differences between treatments.

Table 3.—Per cent repellency of oil based sprays to stable flies for several periods of time following the application.[a]

Formulation	Per Cent Repellency	
	Hours After Treatment	
	9, 10, 11, and 12	28
2% Tabutrex	73.2	75.8
5% Tabutrex	77.3	78.3
2% R-11[b]	63.7	42.1
2% R-326	68.8	21.5
20% Tabutrex	84.2	80.2
2% Tabutrex emulsion (4 oz.)	59.0	40.7
L.S.D. at 5% level	18.715	22.95
at 1% level	26.61	32.64
Average number of stable flies on untreated animals		
	26.6	28.3

[a] Groups of three cows were sprayed with 60 ml. per cow at 6:30 A.M.
[b] 2,3,4,5-bis (Δ_2 butylene) tetrahydrofural.

Experiments conducted during the summer of 1956 were designed to measure the residual repellency of oil solutions applied at a rate of 60 ml. per cow. In three replicates of three animals each, spray was applied at 6:30 A.M. and fly counts were tabulated 9, 10, 11, 12, and 28 hours after application. Since there was little change in relative repellency between the 9th and 12th hours, these figures were combined as an average and listed with 28-hour average figures in table 3.

Weather conditions were warm (85 to 95° F.) and humid for the 2 weeks during which the tests were conducted. These conditions may account for the persistence of Tabutrex (di-n-butyl succinate) repellency through the 28-hour period and the breakdown of R-326. Laboratory aging tests at high relative humidities indicated a similar persistence of Tabutrex and breakdown of R-326. Laboratory tests show that this weakness of R-326 is largely overcome by the addition of a pyrethrins synergist. It may be found that other formulations of oil or large dosages of R-326 emulsions can remedy the weakness and give long-lived repellency with R-326. More work needs to be done with formulations of R-326.

In one test involving a comparison of 2% Tabutrex and 1% Tabutrex with 3% oleic acid, the spray containing oleic acid gave a significantly longer period of animal protection against the stable fly (table 4).

To determine the practical value of the several repellents studied, experimental formulations were supplied to

Table 4.—Per cent repellency of oil solutions to stable flies on cows sprayed with 60 ml. at 6 a.m.

Formulation	Hours After Treatment		
	8	12	28
1% Tabutrex and 3% oleic acid	81.6	78.5	73.0
2% Tabutrex	84.6	70.7	51.8
Average number of stable flies on check cows			
	16.9	19.1	17.4

eight dairy farmers with equal quantities of a standard base oil spray containing 0.03% pyrethrins and 0.3% piperonyl butoxide. The herdsmen were instructed to spray one-half of the herd daily with the experimental formulation and the other half with the standard base oil spray. A dosage of 2 ounces per cow was recommended, but measurements of quantities consumed in the applications showed that the average dosage was only about ½ to 1 ounce per cow. The animals treated with the standard base oil spray served as a check in calculations of percentage repellency. Applications were made for 20 to 30 days, and 20 stable fly counts were made during afternoons.

Results shown in table 5 perhaps do not reveal significant differences among the experimental formulations; however, they do demonstrate that even the herdsmen who do not follow directions for usage can benefit significantly through better fly control obtained by application of the newer repellents.

TOPICAL APPLICATION OF REPELLENTS.—Three experiments were conducted to determine the relative effectiveness of repellents applied topically to cattle by means of a soft cotton pad. In each experiment 30 ml. of pyrenone spray containing 10% piperonyl butoxide and 1% pyrethrins and 30 ml. of 5% Tabutrex with 13% oleic acid were applied to each of three cows, table 6. Observations were made at several periods following application. These experiments were complicated by the fact that 31 animals in the same barn were being sprayed with two other sprays, some of them with a 5% Tabutrex spray and the others with a 0.15% pyrethrins spray.

The number of flies present on the cows topically treated with Tabutrex increased during the first few hours following the use of the 5% Tabutrex and the 0.15% pyrethrins sprays on the other cattle in the barn. This was unexpected and unusual since the number of flies remained low on animals treated topically with pyrethrins. To arrive at an explanation for this phenomenon, a number of laboratory tests were conducted in which adult flies were exposed to space sprays and then transferred to the turn-table cage designed to measure the effectiveness of of repellents. The treated flies apppeared to have lost their ability to discriminate between treated and untreated surfaces. After a period of one or more hours the flies again became sensitive to the repellent. This loss of perception was not only produced by several repellent sprays but also by low dosages of toxicants.

Desensitization appears to be the simple and logical explanation. The fly becomes desensitized or loses discrimination when its sensory receptors are covered over with an irritant. The fly's discriminatory sense is regained when the sensory receptors are freed from the irritant, either by absorption and metabolism or by volatilization of the chemical.

Failure to sort out repellent from non-repellent materials during screening procedures may well be attributable to desensitization of flies, especially if a material is highly irritating or extremely repellent. It could most readily occur in a restricted environment where minute quantities in the liquid or gaseous phase would elicit the maximum sensory response and no gradation of stimulation would be possible. Laboratory tests are currently being conducted to provide more information on the subject.

Proper formulation of spray material can prevent desensitization. Materials which have been useful in this respect are quick-acting insecticides such as Thanite, Lethane 384, allethrins, pyrethrins, and even methoxychlor. A proper balance between the repellent and the killing agent will result in a spray which will kill flies by contact with the spray mist and give a high level of repellency for one or more days.

Table 5.—Average per cent repellency of experimental oil based sprays to stable flies when applied to one-half of each herd by the farmer cooperator at a rate of application varying from ½ to 1 ounce per cow for 20 to 30 days.[a]

HERD NUMBER	NO. OF COWS TREATED	SPRAY FORMULATION	AVERAGE PER CENT REPELLENCY[b]
1	17	2% R-326	70.1
2	12	2% R-326	63.0
3	18	2% R-326	73.6
4	20	2% R-326	60.3
5	9	2% Tabutrex 1% methoxychlor	65.6
6	9	5% Tabutrex 1% methoxychlor	82.3[c]
7	8	AT-25[d]	57.1
8	8	5.2% piperonyl butoxide .5% pyrethrins	60.2

[a] Fly counts were made between 1:00 and 3:00 P.M.
[b] Based upon the number of stable flies found on animals sprayed with 0.03% pyrethrins and 0.3% piperonyl butoxide.
[c] Significant at the 5% level but may have been due to better application by farmer.
[d] AT-25 contained 40% butoxy polypropylene glycol, 2% piperonyl butoxide, and 0.2% cyclethrin.

Table 6.—Stable fly count on cows treated topically with 30 ml. of oil based spray formulations.[a]

HOURS AFTER APPLICATION	AVERAGE NUMBER OF STABLE FLIES PER COW: Pyrenone T-143[b]	5% Tabutrex, 13% Oleic Acid	Check	TEMPERATURE, ° F.
2	0	0.6	17.3	72
6	0	0	22.3	75
24[c]	0	22.2	27.3	68
27	0	11.0	33.0	71
47	6.2	2.7	24.6	76
53	10.8	1.3	30.1	80
72[c]	4.5	8.4	27.3	81
76	5.8	6.0	32.0	84
96	12.0	32.0	35.9	80
100	18.4	28.0	29.0	82
0.75[c]	0	16.4	21.0	85
2.75	5.5	8.2	23.3	90
4.75	0	0	29.0	83
24	8.7	4.0	19.0	87
96[c]	6.6	12.3	23.0	80
100	19.7	0	32.6	89
4	1.3	0	17.4	92
24	4.8	2.6	18.4	95
27	8.3	5.8	23.4	96
48	11.7	3.6	18.0	93

[a] Stable fly counts at various intervals following application gives evidence of desensitization which was caused by spraying the other cattle in the barn with 5% Tabutrex and a spray containing 0.15% pyrethrins with 0.75% piperonyl butoxide.
[b] Mixture of pyrethrin and piperonyl butoxide.
[c] Spraying of other cattle was completed 30 to 45 minutes prior to these counts.

Table 7.—Per cent repellency of various emulsion formulations to stable flies.[a]

Emulsion Concentrate	Dilution	Quantity per Cow	Per Cent Repellency During Days				
			1	2	3	4	6
20% Tabutrex	1:9	1 qt.	82.4	70.3	51.4	—	—
10% Tabutrex and 30% oleic acid	1:9	1 qt.	94.8	90.5	83.7	66.6	—
10% Velsicol AR-50							
20% Tabutrex	1:4	1 qt.	88.3	91.9	79.2	40.0	46.4
10% Tabutrex	1:4	1 qt.	100.0	97.3	95.6	74.0	82.1
30% oleic acid							
10% Velsicol AR-50							
20% Tabutrex	1:9	1 pt.	60.2	21.7	—	—	—
20% Tabutrex	1:9	8 oz.	52.7	17.0	—	—	—
20% Tabutrex	1:9	4 oz.	43.4	12.1	—	—	—
10% piperonyl butoxide							
1% pyrethrins	1:9	1 qt.	91.5	82.3	62.6	32.1	—

[a] The formulations were used at different dilutions and at different quantities per cow. Fly counts were made 10 A.M., 12 m., and 2 P.M. daily.

EXPERIMENTS WITH EMULSIFIED REPELLENTS.—An experiment was devised to establish if possible the most effective formulation and dosage for residual repellency to stable flies on cattle. Groups of three cows were sprayed with formulations at dilutions of 1:4 and 1:9. The emulsions were applied at a dosage of 4, 8, 16, and 32 ounces per cow as shown in table 7. Fly counts were made at 10:00 A.M., 12:00 m. and 2:00 P.M. daily after application.

Results indicated that a dosage of 1 quart of a 1:9 dilution of 20% Tabutrex compared favorably with the quart applications of 1:9 dilution of the activated pyrethrin concentrate. Dosages of 4, 8, and 16 ounces of the same Tabutrex formulation did not produce satisfactory repellency. The formulation containing 10% Tabutrex, 30% oleic acid and 10% Velsicol AR-50, diluted 1:9, was slightly better than the pyrethrin emulsion. The two Tabutrex formulations diluted 1:4 with water and applied at the rate of 1 quart per animal gave excellent residual repellency to the stable fly. The presence of oleic acid in the formulation approximately doubled the residual life of Tabutrex.

EMULSION FORMULATION EXPERIMENT.—Tests were made to determine the effectiveness of the application of various combinations of solvents, repellents, and insecticides in the control of stable flies. All concentrates were diluted 1:4 with water and applied at the rate of 1 quart per cow. Three cows were treated with each formulation and fly counts were made at the time intervals indicated in table 8. Rain early on the fourth day and additional rain on the fifth day caused the experiment to be terminated with the fourth day's fly count. The temperatures during these tests rose to 90 to 92° by mid-afternoon of each day. Unfortunately the stable flies were not so numerous as was desired for a valid comparison of effectiveness.

The results presented in table 8 are interesting, since they indicate that the addition of oleic acid or butoxy polypropylene glycol significantly prolonged the effectiveness of the Tabutrex formulations. A similar advantage was realized when oleic acid was added to Tabutrex oil-base sprays. The addition of methoxychlor was only slightly beneficial in these tests. However, inasmuch as methoxychlor is an excellent toxicant for the control of horn flies, a practical commercial formulation should probably contain this toxicant where horn flies are a problem.

STABLE FLY RESPONSES TO ANIMALS TREATED WITH REPELLENTS.—During the first day or two, cattle treated with Tabutrex repelled flies by what was called a "touch and go pattern." By this is meant that stable flies landed upon the animal and took off immediately. When the temperatures were above normal (90 to 100°) this same material apparently repelled stable flies to a considerable degree by a vapor phase. Flies made an approach to alight, but veered off sharply before they could touch the animal.

Emulsion treatments which have aged 2 to 3 days often exhibit a delayed action type of repellency. The stable fly alights, walks around upon the hairs seeking a desirable feeding site, and then is suddenly repelled when he attempts to push his proboscis down to the skin. This observation led us to believe that much of the residual repellency could be attributed to the irritating barriers of repellent next to the skin of the animal. The repellents were considered effective as long as stable flies were not actually taking blood from the animals. Therefore, fly counts were based upon the numbers of flies that were feeding upon the cows.

SUMMARY.—Several new fly repellents were applied to cattle to determine their relative merits in protecting cattle against stable flies (*Stomoxys calcitrans* (L.)). Repellents R-326 (di-n-propyl isocinchomeronate) and

Table 8.—Per cent repellency of various emulsion formulations to stable flies through 80 hours.[a]

Emulsion Concentrate	Hours After Treatment					
	6	30	52	56	58	80[b]
15% Tabutrex, 20% oleic acid, 15% Velsicol AR-50	100.0	94.6	100.0	100.0	82.7	00.0
10% Tabutrex, 40% oleic acid, 10% Velsicol AR-50	93.9	100.0	100.0	96.8	100.0	100.0
20% Tabutrex	100.0	100.0	100.0	93.6	69.0	00.0
20% Tabutrex, 10% methoxychlor	92.0	100.0	100.0	96.8	88.8	40.7
20% Tabutrex, 20% methoxychlor	100.0	93.3	100.0	86.2	86.2	00.0
50% BPG[c], 10% Tabutrex	100.0	100.0	100.0	83.0	88.8	81.4
10% Tabutrex, 20% BPG, 10% methoxychlor	100.0	94.6	89.2	89.3	100.0	70.2
	Average number of stable flies on untreated cows					
	16.3	24.0	9.3	9.4	11.6	14.0

[a] The formulations were diluted 1:4 with water and applied at a rate of 1 quart per cow.
[b] Counts made after animals were exposed to 0.5 inches of rain. Maximum temperatures of 90 to 91° F. during these tests.
[c] Butoxy polypropylene glycol.

Tabutrex (di-n-butyl succinate) used in base oil sprays gave better economic control of stable flies than activated pyrethrins. Tabutrex oil solution or emulsion formulations gave 1 to 6 days residual repellency to the stable fly.

Flies lost their ability to discriminate between treated and untreated animal surfaces after an exposure to mists containing the repellents used in the tests. After an interval of time the flies regained their sense of discrimination.

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A Residual Spray for Control of the Periodical Cicada[1]

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Brood XIV of the periodical cicada, *Magicicada septendecim* (L.), appeared in Washington County in Western Maryland during 1957. This was a much smaller brood than Brood X which infested all of the State in 1953. There were only three or four orchards damaged by Brood XIV, but the population in these orchards was extremely heavy.

DAMAGE.—The adult cicadas began to emerge from the soil around the 15th of May, and within a few days after emergence the females began puncturing twigs and depositing eggs. These punctures weakened and caused severe breaking of twigs on both large and small trees, and in many instances trees from 1 to 2 years old were too severely damaged to recover. Figure 1 shows a young peach tree damaged by the punctures. Figure 2 shows the same tree after most of the punctured twigs were removed.

CONTROL.—In 1936 when Brood X of the periodical cicada occurred in Maryland, the senior author (unpublished) found that a pyrethrum spray was highly effective against the adult cicadas, and several hundred gallons of the material were used in commercial orchards in that year. Later Woodside (1948), Cutright (1949), Asquith (1952), and Graham & Cochran (1953) found that tetraethyl pyrophosphate was effective in killing the adult cicadas when they came in contact with the spray, but that it had no residual effect and, therefore, the applications had to be repeated at 3- or 4-day intervals in order to prevent severe damage to the trees. During the 1953 outbreak, many orchards in Maryland and adjoining states were sprayed with from one to six applications of tetraethyl pyrophosphate, and in many instances the results obtained in Maryland orchards were disappointing. Tetraethyl pyrophosphate is highly toxic to

FIG. 1.—Young peach tree damaged by cicadas.

FIG. 2.—The same tree after most of the damaged limbs were pruned.

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Table 1.—Mortality of the periodical cicada at 24-hour intervals caged on trees before the spray was applied.[a]

Materials and Amounts per 100 Gallons	Number Dead at End of Each 24-hour Period		
	24	48	72
Trithion 25%, WP 1 lb.	3	6	18
Tedion[b] 25%, WP 0.5 lb.	0	5	25
Chipman R-6199, 3 oz.	2	7	19
Nialite[c] 25%, WP 1 lb.	6	7	21
Phosdrin,[d] 1 pt.	25	—	—
Guthion 15%, WP 1 lb.	5	6	12
Sevin[e] 50%, WP 2 lb.	25	—	—
Sytam,[f] 1 qt.	0	2	8
Check	0	2	9

[a] Each cage contained 25 cicadas.
[b] Comprised of 80% 2,4,5,4-tetrachlorodiphenyl sulphone,
[c] Methanedithiol, bis (0,0,diethyl phosphorodithioate).
[d] Contained 2 lbs. of Phosdrin insecticide per gallon.
[e] N-methyl-1-naphthyl carbamate.
[f] Contains 4 lbs. schradan per gallon, Schradan, 42%; tris (dimethylamino) phosphine oxide, 15%.

the operator, injurious to the fruit and foliage, and costly when several applications are required to give control. The only sure method of control prior to 1957 was to wrap young trees with cheese cloth, but this method was practical only on small trees. Trees over 3 years old are very difficult to cover, and the cost of the material and labor involved in wrapping is prohibitive.

After the appearance of Brood XIV in 1957, a series of experiments was carried out to test the effectiveness of some of the new insecticides that have been developed since Brood X occurred in 1953. Eight plots in an apple orchard of 7-year-old Jonathan trees consisting of 16 trees per plot were used in the original test. Before the spray was applied, 25 cicadas were collected and placed in a screen-wire cage which was then hung in the center tree of each plot. After the sprays were applied, three examinations were made at 24-hour intervals to determine the mortality in each cage. A check cage was hung outside of the sprayed area and examined at the same intervals. The materials used and results obtained with each material are shown in table 1.

A study of table 1 shows that only two of the materials, Sevin and Phosdrin, gave 100% kill in the first 24 hours after the spray was applied. The other materials gave variable results. Twenty-four hours after application of the insecticides in this test great numbers of moribund cicadas were observed around the trunks of trees and on weeds under trees treated with Sevin. Observations on all other plots, including the Phosdrin-treated plot, showed that the trees and weeds were heavily infested with normal cicadas. It appeared that the insecticide, Sevin, had a definite residual effect against the cicadas. In order to determine the duration of the residual effect, and the time required to kill cicadas coming in contact with the foliafe, several large cages were constructed for use in additional tests. Each day 100 cicadas were collected from an area that had not been sprayed and placed in a cage attached to a limb on a sprayed tree, as shown in figure 3. A similar cage placed on a tree outside of the sprayed area was used as a check and a third cage was placed on a tree in the plot sprayed with Phosdrin. Examination of the latter cage after 72 hours showed that only six cicadas

FIG. 3.—Examining cage for dead cicadas.

were dead, and therefore no further work was done with this material. The cages on the plot sprayed with Sevin were examined every 24 hours to determine the number dead. A study of table 2 shows that Sevin remained 100% effective for 6 days after the spray was applied, May 30 to June 4. It continued to give a high degree of kill 2 days later after which time the observations were discontinued.

After it had become apparent that Sevin had a residual effect against the cicada, two large plots, each consisting of 90 trees including the following varieties, Jonathan, Stayman, Rome Beauty and Grimes Golden, were selected for large scale tests. One block of these trees was sprayed with a dilute application of 2 pounds of Sevin to each 100 gallons of water, and the other was sprayed with 6 pounds to each 100 gallons of water (3× concentration). These

Table 2.—Residual effect of Sevin on the periodical cicada sprayed on May 29 with a concentration of 2 pounds to 100 gallons of spray.

Cage Number[a]	Date Placed	Number Dead at End of Each 24-hour Period				
		24	48	72	96	120
1	May 30	17	100	—	—	—
2	May 31	31	98	100	—	—
3	June 1	22	57	94	100	—
4	June 2	23	57	74	89	100
5	June 3	23	53	98	100	—
6	June 4	15	68	75	[b]	100
7	June 5	0	4	[b]	57	83
8	June 7	14	[b]	28	49	52
Check		1	6	6	6	15

[a] Each cage contained 100 cicadas.
[b] No record taken.

applications were made with an airblast machine delivering 62,000 cu. ft. of air per minute. Both blocks were heavily infested at the time the applications were made. Observations, 6 hours after the spray was applied, showed that the trees were practically free of the pest, and literally thousands of the cicadas were buzzing in the cover crop beneath the trees. Observations in these two blocks during the next 3 weeks failed to reveal any cicadas on the trees, but each day they could be found in the cover crop around the tree unable to fly.

In another part of the orchard a single tree of Grimes Golden, surrounded on three sides by other trees and on the fourth side by woods, was sprayed with Sevin. A canvas sheet, which covered approximately one-half of the of the area of the tree, was spread beneath it before the spray was applied. Daily observations of this tree failed to reveal any live cicadas on it for 6 days after the spray was applied, and during this period dead cicadas were collected from the sheet each day, totaling 570 during the 6-day period. Further observations showed that after 6 days the tree became reinfested, and at the end of 10 days no difference in infestation could be observed between this and the adjacent trees. The material, either in the dilute or 3× concentrate, did not appear to injure the fruit or foliage of the varieties used in these tests.

SUMMARY.—Results of experiments conducted at Hancock, Md., show that the insecticide Sevin had a long residual effect against the periodical cicada (*Magicicada septendecim* (L.)). In small scale tests this material used at the rate of 2 pounds to 100 gallons of water gave complete control of cicadas emerging from soil around the trunks of the sprayed trees and also those flying in from adjacent areas for a period of 6 days. It remained partially effective up to 10 days. Sevin gave 100% kill of cicadas caged on the limbs of trees for 6 days after treatment and remained highly effective for 10 days.

In plots consisting of 90 trees each one sprayed with Sevin at the rate of 2 pounds to 100 gallons of water and another sprayed at the rate of 6 pounds to 100 gallons, both dosages gave complete control of cicadas for a period of 3 weeks.

There was no apparent injury to the fruit or foliage sprayed with Sevin at 2 or 6 pounds per 100 gallons of water.

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Concerning the Harmlessness of Insect Pathogens and the Standardization of Microbial Control Products[1]

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The renewed interest, during recent years, in the use of microorganisms as a means of controlling insect pests has engendered some concern in certain quarters as to the safety of these agents for man. Are they really as harmless (to forms of life other than insects) as insect pathologists seem to think? Does the application of these insect pathogens on food crops leave residues of any danger to human beings or to vertebrate animals that may consume these crops? Do microbial control products in any way harm the crops themselves? Is there significant danger in the possibility that these microorganisms, being living agents, might mutate or change into organisms pathogenic for man, livestock, or plants? Are the insect pathogens used against pest insects directly harmful to or pathogenic for beneficial insects such as bees and insect parasites and predators?

On the basis of available knowledge, the first of these questions may be answered in the affirmative, and the last four, with possible minor exceptions, may be answered confidently in the negative. It would appear wise, however, to spell out somewhat more clearly than perhaps has previously been done, the reasons for this confidence. It is especially important that this be done now that commercial concerns both in the United States and in Europe have shown interest in expanding the manufacture and marketing of microbial control products. Furthermore, in conjunction with assuring the safety of these products, there needs to be a thorough consideration of methods of determining standards for their potency and purity, and, of deciding what, if any, tolerances should be established. Therefore, at this point in the development of microbial control methods it might be well to consider certain background information and some broad aspects of these matters.

THE HARMLESSNESS OF INSECT PATHOGENS.—The author (Steinhaus 1956) has recently outlined the advantages and disadvantages of microbial control methods as compared with other means of controlling insects. Included among the list of advantages was the assertion that while lethal for insects, entomogenous microorganisms are harmless for man, animals, and plants. The bases for this statement may be stated rather simply.

[1] Prior to and during the preparation of this article, the author consulted with and received valuable advice from several individuals to whom he owes thanks. These include R. Z. Rollins, Chief of the Bureau of Chemistry, California State Department of Agriculture; W. G. Reed, Head of the Pesticide Regulation Section, Plant Pest Control Division, U. S. Department of Agriculture; and H. A. Chittick, Fairfax Biological Laboratory, Clinton Corners, N. Y. Accepted for publication August 29, 1957.

In the first place, we know of no authenticated recorded instance of a true insect pathogen having caused an infectious disease in a vertebrate animal either experimentally or in nature.[2] Infectivity tests in laboratory animals using true insect pathogens have so far revealed no serious indication that they are capable of infecting vertebrates. (Contrariwise, it has been well demonstrated that the classical pathogens of man and higher animals are generally nonpathogenic for insects; thus indicating the wide gap in the range of susceptible hosts.) Furthermore, although microorganisms are not yet widely used in the field (largely because of the commercial unavailability of suitable products), there nevertheless has not been a single reported instance of harm to plants or higher animals as the result of numerous experimental field trials by investigators in many parts of the world since just before the turn of the century. In the second place, most of the true insect pathogens are highly specific, capable of infecting only a small group of insects, or even a single species. Thirdly, in general entomogenous microorganisms are biologically not the type, nor do most of them belong to the groups, that are known to cause disease in higher animals or in plants. Fourthly, outbreaks of disease among insects in nature have shown invariably that the disease remains confined to insects, and is not transmitted to the host plants or to animals feeding on the infested plants.

The possibility of harmful mutants arising during the use of entomogenous organisms must, of course, be considered. From the evidence at hand, however, the likelihood of harmful mutations occurring appears to be remote. During the past century's work with insect pathogens, no such mutants have been reported. Furthermore, on the basis of present-day knowledge of microbial genetics one would not expect such transitions to survive and to escape rapid detection. The situation should not be confused with what happens when avirulent strains of known vertebrate pathogens suddenly give rise to virulent strains. For example, the likelihood of insect viruses, at their present stage of evolutionary development, becoming pathogenic for man is probably as remote as is the likelihood of the plant viruses that multiply in insects becoming pathogenic for man.

There is, however, still more to be said in this connection; it has to do with the popular concept of the place of microorganisms in man's daily life. Any excessive fear that because insect pathogens are living microbes they are therefore inherently dangerous and their use not to be contemplated, indicates an erroneous type of thinking and an inadequate understanding of microbiological and agricultural sciences especially as they pertain to other beneficial uses of microorganisms. There is as much reason to fear harm by ladybird beetles or silkworms simply because they are insects (as are the destructive Japanese beetle or the European corn borer) as it is to fear infection by a microsporidian because it is a protozoan (as is the malarial parasite). It is similarly unreasonable to fear (and fortunately most people do not) those plant-infecting viruses, bacteria, and fungi that are consumed daily along with fresh vegetables and other plants.

Consider, for a moment, that of the thousands of different species of microorganisms known to man, and distributed ubiquitously in his environment, only a small number are what we might call harmful or dangerous to him. By far the majority of "germs" are either beneficial or absolutely harmless. Without them this earth would become heaped with refuse and debris, making life as we know it today impossible. To these minute organisms we owe the decomposition of waste matter and the fertility of our soils. Without them we would not be able to prepare our food as well as we do today. We need or have needed them to help make our cheeses, cultured milks, butter and bread, our sauerkraut, pickles and olives, our vinegar, wines, and other alcoholic beverages. We may make use of them in curing tobacco, tea, and leather. Similarly, microbes may play a part in preparing for market such commodities as coffee, cocoa, and soybean sauce. Not only do microbes play an important role in the manufacture of food for humans, but they also find a place in the production of silage for livestock. If, in addition, we consider the many industrial products and medicinals manufactured with the aid of microorganisms it is easy to see that these agents are our friends much more frequently than our enemies. Indeed, in spite of the tremendous strides made through the applications of chemistry in industry and in our daily living, the exploitation of microorganisms by man goes on at an unbounded rate. Microorganisms are finding an ever increasing use in the chemical industry itself. Is it not logical and in keeping with scientific progress, therefore, that every effort be made to exploit micoorganisms for whatever aid they can give us in the control of insect pests? It is probably true, theoretically and basically, that most such problems are amenable to a solution on the basis of chemistry. But, just as in industry, it is frequently the case that microorganisms may serve as the most economical, most effective, and most convenient purveyors of chemical agents or reactions.

These points have been raised to emphasize how unwarranted it is to fear certain microbes that can be used against insects just because they are microbes or just because they may be applied in a manner similar to that used with toxic chemical insecticides. In all likelihood the public would shy away from drinking cultured milks if the manufacturers emphasized the fact that one of the organisms inoculated into the milk and drunk by the consumer was a streptococcus, albeit a harmless one. On the other hand, few would refuse a delicious morsel of Roquefort cheese even though the blue fungus is plainly visible. And then there are those who delight in eating yeast without any concern that they are consuming microorganisms. Many of the microbes consumed in foods are taxonomically closer to known human pathogens than are many of the insect pathogens. As a matter of practical utility, there should be no more apprehension in using entomogenous microorganisms to kill insects than in drinking a glass of buttermilk or eating a piece of Roquefort cheese. In spite of the overall harmlessness of the kind of microorganisms we have been discussing, their use, particularly in foods, nevertheless requires at least a modicum of regulation. For example, all dairy products and other foods produced by fermentation and containing

[2] The literature contains several reports (see MacLeod 1954) of fungi identified as belonging to the genus *Beauveria* (which contains important entomogenous species) that have been isolated from diseased vertebrate tissue. Some of these fungi are insufficiently described, and in no case could they be identified with certainty as typical and true entomogenous species or strains.

microorganisms, are subject to the Federal Food, Drug, and Cosmetic Act when they are shipped in interstate commerce. Similarly, the interstate distribution of nitrogen-fixing bacteria used in the cultivation of leguminous crops have been subject to U. S. Department of Agriculture regulations which were enforced up to 1944. Many states have specific laws and conduct active enforcement programs on these products.

Another aspect to remember is that even if insect pathogens were potentially pathogenic for higher animals and plants, it would be no more justifiable to rule out categorically their controlled use than it would be to rule out the proper use of highly toxic chemical insecticides. Fortunately, however, insect pathogens are not comparable in this respect to chemical insecticides, and thus enjoy an advantage as far as hazard to health is concerned.

Insect pathogens may be utilized in several ways. They may be introduced into a population of insects and allowed to spread unaided. They may be used as sprays or as dusts. They may be used in conjunction with chemicals or with entomophagous insects. Regardless of the method of application, what we have stated concerning their harmlessness applies. There is no known fundamental reason to fear direct danger to crops, livestock, or to man as the result of microbial residues. Ordinarily, the likelihood of danger to the person applying microbial insecticides is also slight, but certainly care should be exercised to avoid swallowing or breathing massive doses of the microbial agents concerned. Undoubtedly, most insect pathogens could be consumed by man without harm, but to do so is unnecessary and hence to be avoided as one would wish to avoid consuming large quantities of many ordinary saprophytic organisms met with in our daily lives. It is true that "cocktails" of some of the insect pathogens have been purposely consumed by humans to demonstrate their harmlessness, but this would not be a wise standard procedure except with an ardent insect pathologist wishing to press his point. Not to be forgotten is the possibility that an occasional individual might be allergic to a particular microbial agent, as might be the case with virtually any proteinaceous substance.

The effects of disease on such density-dependent control factors as insect parasites and predators have been discussed elsewhere (Steinhaus 1954). Suffice it here to say that although there are some insect pathogens capable of infecting both the host insect and its insect parasites, such instances are not common, and so far the occasions when this interferes with the use of microbial insecticides in the field are rare. Fortunately, the relatively high specificity of most insect pathogens makes it very unlikely that serious trouble will occur during practical field use. Such important insects as bees, for example, appear to be generally immune to most entomogenous bacteria, viruses, and protozoa tested against pest insects. (The same may be true of the fungi, but more tests are necessary before full assurance can be given on certain of these organisms.) However, because of the economic importance of the honey bee, as well as other bee pollinators, it would be wise to test the susceptibilities of these insects to any new product which, on the basis of its biological attributes, would appear to be potentially pathogenic for them. In this connection, it should be remembered that one of the advantages of microbial control over chemical control is the fact that entomogenous microorganisms can usually be used concurrently with entomophagous insects.

LEGAL ASPECTS.—There has been some question whether federal and most state laws regulating the use and marketing of chemical insecticides actually apply to microbial control products. Basically, there may not be a great deal of difference between marketing a microorganism to kill an insect and selling a cat to catch mice; but on more careful analysis this analogy appears somewhat unrealistic, especially in a highly organized and commercialized society such as ours. It may also be argued that inasmuch as microorganisms are living beings [we are including viruses here], and are essentially parasites of insects, they no more come under the regulations governing insecticides and economic poisons than those insects that parasitize or are predators on insect pests. If size alone is a criterion, where is the line to be drawn between the minute entomophagous insects, the entomophilic nematodes, and the larger microbial pathogens? They are all living entities and are all parasites of insects. Therefore, if insect parasites do not require regulation by the pesticide branches of federal and state agencies, why do microbial parasites?

In fact, the introduction and dissemination of insect parasites and predators are regulated by legislative acts, namely the quarantine laws; but acts pertaining to chemical insecticides and economic poisons are generally regarded as not applicable to entomophagous insects. And, this distinction seems justified. Moreover, it must be remembered that insect parasites and predators have a negligible overall potential of danger to man, animals, and crops, whereas microorganisms as a complete group admittedly include species detrimental to man's health and interests, although, of course, entomogenous species are not among these. Nevertheless, in many respects, it might appear justifiable that those measures regulating the introduction, distribution (marketing) and use of entomophagous insects are sufficient, fundamentally, for similarly regulating the use and marketing of entomogenous microorganisms.

To be sure, microbial control products are frequently used and disseminated in a manner similar to that of other pesticides. Considering them as such by federal and state agencies would provide a type of legal control that would facilitate the orderly marketing of effective products. On the other hand, there are occasions on which pathogens may be "introduced" into a population of the pest insect in a manner similar to that used in cases of introducing parasites and predators into host populations. Under these circumstances would federal and state officials consider them in the manner of pesticides or microbial parasites? Our examination of pertinent laws and regulations, as well as consultations with such officials, leaves with us a residue of some doubt and ambiguity on this particular question. On the other hand, there appears to be no question that the U. S. Department of Agriculture currently holds the view that commercial products containing microorganisms (including viruses) as active ingredients, and distributed interstate, with the functional objective of pest control, are economic poisons subject to all of the provisions of the Federal Insecticide, Fungicide, and Rodenticide Act.

The Federal Insecticide, Fungicide, and Rodenticide

Act defines the term "economic poison" as "any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any insects. . . ." In attempting to interpret this definition in the light of the problem being discussed, it might be asked what is meant by the word "substance." Are microorganisms (i.e., microbial parasites), or their spores, "substances"? Are parasites and predators "substances"? Not according to Webster's New International Dictionary, or according to the generally accepted use of the term. The same difficulty with the word "substance" pertains to the Act's definition of the term "insecticide" which is defined as "any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any insects which may be present in any environment whatsoever." In this case, however, a broad definition of the word "insecticide" (*insectum*, insect + *-cide*, to kill) would include living organisms as well as anything else that could be used to kill insects. (As defined by Webster's Dictionary, an insecticide is "an agent or preparation for destroying insects.")

State laws and codes may vary somewhat in what they consider an economic poison, insecticide, or pesticide, but in general most of them encompass approximately the same principles as the federal act. The same may be said of most municipal codes. In the case of the Sanitary Code of the City of New York, however, one finds the following regulation:

> "No person shall prepare or manufacture, sell, offer for sale, give away, deal in, supply, or use, or have in his or her possession with intent to sell, offer for sale, give away, deal in, supply, or use, exterminator or insecticide which contains living bacterial organisms or any culture or preparation thereof."

Such a requirement would appear unnecessarily to deprive the citizens of New York of promising safe control measures as far as insect pests are concerned. The regulation probably grew out of restrictions pertaining to bacterial rodenticides which may be in quite a different category from bacterial insecticides. The pathogens applicable against rodents could be of the type that, under certain circumstances, cause infection in man. This is not the case, however, with insect pathogens which have a much more limited and restricted host range. From the standpoint of the hazards involved, the two situations are definitely not parallel.

At the present time, in the United States, one important type of microbial control product is on the market, and is regulated by federal and state agencies. This product consists of the spores of *Bacillus popilliae* Dutky and *Bacillus lentimorbus* Dutky suspended in an inert powder (talc and precipitated calcium carbonate). The bacteria concerned are the cause of milky diseases in the Japanese beetle, *Popillia japonica* Newm. The product is marketed under the name "Doom Milky Disease Spores" (formerly under the name "Japonex"). At one time another similar product was sold under the name "Japidemic."

These products have been marketed under licenses granted by the U. S. Department of Agriculture which holds the pertinent patents. All licensees are required to meet the specifications established by the Department of Agriculture; essentially these specifications require that the product contain 100,000,000 viable spores per gram of material. Because of the nature of the product and the manner in which the spore concentrate is prepared it is very difficult to state the percentage of active ingredient by weight, as is the case with chemical insecticides. Incidentally, microbial control products containing bacterial spores have, in the past, been marketed in Europe (*e.g.*, "Sporeine"), and, as in this country, consideration is presently being given to the commercial sale of similar products.

The federal Food and Drug Administration (hereinafter referred to as FDA) which, under the Miller Amendment, has the obligation of establishing the official tolerance of a pesticide on a particular crop, and of deciding whether a pesticide may be exempted from the requirement of a tolerance. Some pesticide chemicals (*e.g.*, sulfur, lime, sodium carbonate) have been declared safe, thus requiring neither a formal tolerance nor an exemption. In which, if not all, of these categories microbial control products are likely to be considered is not yet predictable. On the basis of evidence (that they leave no toxic residues) now at hand and reported in the literature it would appear reasonable to expect them to be exempted from tolerances, or to be declared safe. Ordinarily, an exemption from a tolerance is granted "when it appears that the total quantity of the pesticide chemical in or on all raw agricultural commodities for which it is useful under conditions of unrestricted use will involve no hazard to the public health." Again, all available evidence indicates that entomogenous microorganisms come well within these limitations. However, since petitions must be submitted to the FDA for exemptions, as well as tolerances, and since no such petitions are now required for the use of entomophagous insects, it is hoped that microbial parasites will be considered in the same manner and be declared safe.

On the other hand, the convenience gained by this avoidance of red tape would be slight compared with the advantages gained for the promotion of microbial control materials, as well as applied insect pathology generally, by complying with every regulation, act, or code that would in effect attest to the harmlessness of insect pathogens for other forms of life.

There is no good reason why, for every new microbial insecticide placed on the market, a full report with respect to the safety of the product should not be provided the FDA. As is required for pesticide chemicals, this report should, where necessary, include data derived from appropriate animal or other biological tests and experiments. Thus confidence in the product from the standpoint of safety would be assured the user, the consumer of the plants treated, the manufacturer, and the state and federal regulatory officials.

With the advent of the commercial availability of microbial insecticides, there should be no question that they be required to meet certain standards of potency, requirements of purity, and guarantees of safety. It is essential, however, that these requirements be realistic. Although it is true that in certain respects, such as in methods of application, living microbial insecticides are similar to chemical insecticides, there are important differences between them that should be appreciated by everyone concerned.

ESTABLISHMENT OF SAFETY STANDARDS.—In light of the convincing evidence indicating the harmlessness of insect pathogens for higher animals, is it necessary, before marketing commercial preparations, to test the potential pathogenicity of these microorganisms for animals? The only prudent answer to this question is definitely an affirmative one. This is especially advisable and necessary in the case of those insect pathogens that are to be marketed as suitable for treating food or forage crops. Obviously the use of microbial insecticides against insects such as those infesting ornamental plants and forests would be appraised on a different basis. The reason for requiring such tests in vertebrate animals is that it provides a safeguard to the consumer that he has a right to expect, and that it comes within the spirit, if not the letter, of those laws and regulations now in effect regarding insecticides generally. Furthermore, such tests would guard against the possibility of errors in manufacturing which conceivably might permit the incorporation of a harmful contaminant into the product, or some peculiar condition that might cause the insect pathogen to be potentially capable of infecting another form of life. Furthermore, they would serve to protect users and manufacturers from improper practices and unfair competition that would tend to destroy public acceptance of microbial control products and react to the detriment of this promising method of controlling certain insects. For these reasons, therefore, it is better to have such safety tests made before a product is placed on the market even though the material is essentially harmless. The security and assurance such testing provides would not only give the consumer confidence but tends to place microbial control methods generally on a sound basis.

What should be the nature of such safety tests? At the present time, governmental agencies request information on the pathogenicity for warm-blooded animals of any microbial control agent, and on the variations that may be expected from batch to batch in a normal production schedule. The exact nature of these pathogenicity tests, which laboratory animals to use, what dosages to use, etc., have not, to our knowledge, been made a formal part of regulations governing these matters. Perhaps, therefore, it is not presumptuous to suggest here possible tentative standards that might be considered from a practical as well as theoretical standpoint:

(1) The potency, *i.e.*, virulence, of the commercial product should meet known and experimentally proved requirements of effectiveness against the insects for which it is recommended. This potency should be indicated on the label of the marketed product in terms of the amount of active ingredient per unit weight or volume of the material. This may be expressed as the number of viable organisms in a given amount of the product. The exact manner in which this is stated might vary according to the pathogen concerned and diluent or carrier used. Thus, a given product may be said to contain a certain number of viable bacteria, protozoa, fungi (or their spores), or nematodes (stage indicated). The species of microorganism concerned should be included in this statement. In the case of insect viruses, the principal ingredient may have to be indicated in terms of the degree of infectivity per unit volume. With those viruses contained in inclusion bodies (polyhedra, capsules), the number of inclusion bodies per unit weight or volume may serve as a fairly reliable guide to the potency of the preparation. At least, on the basis of present techniques, this would be simpler than attempting to indicate the number of virus rods in a given amount of the product. Thus, a conceivably adequate ingredient statement might read:

ACTIVE INGREDIENT: Spores of *Bacillus thuringiensis* Berliner; 100 million viable spores per gram of inert clay [or other material].

Actually, the federal and most state laws require the label of an economic poison to show either (a) the name and percentage of each active ingredient and the total percentage of inert ingredients, or (b) the name of each active ingredient, the name of each inert ingredient, and the total percentage of inert ingredients. Thus the type of ingredient statement just given does not strictly comply with the wording of most such laws. Of course, it may satisfy the law by adding an arbitrary legend such as "Inert ingredient, clay 99.5 per cent." As was learned during the registering of Japanese beetle milky-disease spores, it would be very difficult and in many cases virtually impossible (because of the manner in which the concentrate is prepared) to express the quantity of "active ingredient" in percentages by weight. In the case of spore powders, the strength of the final product can probably best be stated in terms of the number of viable spores in a given quantity of powder. For nonsporeforming organisms and for viruses a variation of this phraseology, or some other criterion, will undoubtedly be required. In some cases it is conceivable that the strength of the product may be expressed in terms of the amount of microbial toxin present. It is hoped that an appropriate ingredient statement giving the requirement for microbial control products will be incorporated in the federal and state economic poison laws.

(2) Before being placed on the market, each commercial microbial control product should be tested thoroughly and comprehensively at least once for indications of its potential pathogenicity for animals. This might best be accomplished by the peroral and parenteral administration of large doses of the product to appropriate laboratory animals. The doses should not, however, be so large as to cause a reaction on the part of the animal to foreign protein as such. It might be well to use mice, guinea pigs and rabbits for this test. If, on the basis of these tests, any doubts arise as to the nonpathogenicity of the product for vertebrate animals, especially man, monkeys could be included among the test animals. This comprehensive test might include a temperature record of all animals except the mice, and at least a gross pathological examination of the principal organs and tissues of any animals that die, or of representative sacrificed animals. In some instances it might be well to include domestic animals (*e.g.*, chickens and pigs) in the susceptibility test.

Once this comprehensive test has been made, periodic tests, using fewer animals, and fewer species of animals, should be made at extended intervals (or, in some cases, of every batch manufactured) in order to detect pathogenic contaminants, or pathogenic mutants.

There is no evidence to indicate that the periodic tests here proposed need be any more rigid than the safety test

required by the U. S. Public Health Service regulations on biologic products (". . . any virus, therapeutic serum, toxin, antitoxin, or analogous product applicable to the prevention, treatment or cure of diseases or injuries of man"). This safety test is described in Public Health Service Publication No. 473, "Regulation of Biological Products," Revised August, 1956, p. 20, as follows:

"In general, the safety test shall consist of the parenteral injection of the maximum volume tolerated, but not more than 0.5 ml. into mice weighing approximately 20 grams each and 5.0 ml. into guinea pigs weighing approximately 350 grams each. When the injections are made into at least two animals of each species, there shall result neither significant symptoms nor death during an observation period of not less than 7 days. Variations from this test, either in the volume injected or in the species of test animal used shall be made whenever required because of the human dose level demanded of the product or because of any individual demands of the product itself."

It is recommended that the same, or similar, standard be used in periodically testing the safety of microbial control products intended for use in combating insects. It would seem appropriate that these safety tests be completed by the manufacturer, or his agent. It should be required that samples of any lot of the product, together with the protocols showing the results of the tests, be sent to the appropriate federal or state agency for examination at any time they may be requested.

In addition to maintaining standards of potency and safety, other appropriate standards should be required, but specific requirements would depend upon the particular product concerned. For example, any toxic chemicals added to the product should be appropriately regulated, all inert ingredients should meet generally accepted standards of purity and quality, and no ingredient should kill, weaken, or denature the microorganism, or its spores, below the minimum acceptable potency within the dating period when stored at the recommended temperature and humidity. The dating period should be determined with reference to the date of manufacture.

Similar tests could be devised with relation to plants, although this would not appear necessary if no indication of harm to plants showed up during the original experimental plot and field tests. The fact that one or two instances of an entomogenous fungus surviving on certain plants (*Beauveria* on bunch grass and cornstalks) have been observed does not provide any reason for great concern; it simply is an indication that when any doubts arise infectivity tests are always wise from a precautionary standpoint. Of course, if additives, or even chemical insecticides, are incorporated in the product, appropriate toxicity tests on these would be required.

No attempt has been made to suggest here a complete set of regulations governing the manufacture, sale, and use of microbial control products. Instead, we have sought to call attention to what appear to be the principal points of consideration pertaining to the establishment of standards for these products. If these problems are satisfactorily handled, there need be no difficulties in certifying the use of entomogenous microorganisms without fear of their causing any direct harm to man, other vertebrates, or to plants.

SUMMARY.—In general, microorganisms pathogenic to insects, and used as active ingredients in microbial control products, are harmless to man, animals, and plants. Their exploitation as a means of controlling insect pests should not be avoided simply because they are microorganisms, any more than is the use of microbial life in the production of certain foods, fermented products, and in certain industrial processes. Nevertheless, in order to protect the public use and acceptance of microbial insecticides, as well as to protect manufacturers from unfair competition, and to prevent improper practices in general, it is advisable and justifiable that such products be subject to appropriate State and Federal regulations. The relevancy of present laws and regulatory acts to the manufacture and distribution of such products is discussed. Provisionary methods of standardizing microbial insecticides and ensuring safety standards for them are suggested. Standards of potency and safety should be based on an informed scientific viewpoint and maintained in such a way as to fully protect the public, yet not hamper or impede the development of this promising method of insect control.

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BULLETIN OF THE ENTOMOLOGICAL SOCIETY OF AMERICA

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Effect of Insecticide Treatments to Alfalfa on Honey Bees, Including Insecticidal Residue and Honey Flavor Analyses[1]

CARL A. JOHANSEN, MARVIN D. COFFEY, and JOHN A. QUIST[2,3]

Investigations were conducted in the Yakima Valley of Washington in 1953 to find an insecticide which would control pea aphids in alfalfa grown for seed without greatly decreasing the pollination by bees. Results indicated that demeton was the most promising material for this problem (Johansen 1954). Further tests to determine the effects of demeton, schradan, and parathion upon honeybees and the honey which they produced were carried on in 1954 and 1955.

MATERIALS AND METHODS.—Four alfalfa plots, ranging from 2 to 6 acres and surrounded by wheat or other small grains, were set up in the Pullman area of Washington in 1954. Each of these plots was 4 to 14 miles from any of the others and several miles from any suitable honeybee forage crop. Each of these plots, which were considered as replicates, was treated with 1 quart of 22% demeton per 5 gallons per acre. Two other plots were selected for untreated checks. In 1955, ten experimental plots of a similar nature were established in the Pullman area. Four of these were treated with 1 quart 42% schradan per 5 gallons per acre. Four were treated with 1 quart 25% parathion per 5 gallons per acre, and the remaining two were untreated checks.

During both seasons the insecticides were applied by means of a truck-mounted sprayer with a 30-foot boom using 35 pounds pressure. Three to 15 days before treatment, two hives of honeybees were placed in each plot. Selection of one strong hive and one weak hive in each case helped to insure greater uniformity. Immediately after application of the insecticides, two marked frames with drawn comb were placed in each hive.

In 1954, areas approximately 4 sq. ft. in front of each hive were covered with cheesecloth, which was pegged down. Dead bees which collected on this surface after treatment were counted. Sheet metal pans were used for this purpose in 1955 to obtain more positive collection of samples. The pans were 24 by 28 inches with a 3-inch edge on 3 sides and parts of the 4th (see fig. 1). The front cleat of the hive bottom board was placed in the open side of the pan so that the resultant collecting area was 24 inches square.

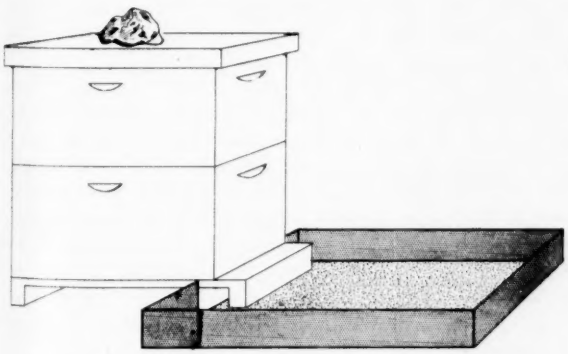

FIG. 1.—Pan used to collect samples of dead honey bees at hive entrance.

Bee activity was observed in each plot after treating. Dead bees in front of the hives were counted at 24- and 48-hour intervals. The marked frames were removed from the hives 1 week and 3 weeks after the alfalfa was sprayed. Pea aphids were checked by taking two 25-sweep samples with a 15-inch sweeping net at 1-day, 3-week, and 6-week intervals. Representative samples of approximately 10 pounds of alfalfa were obtained from each plot at 3-week and 6-week intervals. These were air dried for 1 week, chopped in a feed grinder (with the screens removed), and thoroughly mixed. Sub-samples were taken for insecticidal residue analyses. The number of deep extracting frames of honey produced during the 3-week period following treatments was determined for each hive.

DISCUSSION.—Laboratory tests have shown a feeding type application of demeton is toxic to honey bees (Eckert & Tucker 1954, Jones & Connell 1954). However, field applications appear to present little hazard. It has been suggested by several workers that demeton may be absorbed by plants fast enough so that it does not actually contact more than a small proportion of the honey bee (*Apis mellifera* L.) field force. Atkins & Anderson (1954) found in laboratory tests that demeton dusts were relatively safe to honeybees. Lieberman *et al.* (1954) and Johansen (1954) have concluded that demeton applied to alfalfa when the bees are not foraging is safe. In the tests reported here, honey bees were not adversely affected even by demeton sprays applied to blooming alfalfa at 2:45 p.m., while the bees were actively foraging (table 1).

Feeding tests have shown schradan to be highly toxic to bees (Johnsen 1955). However, as with demeton, field applications in this study caused little, if any, bee poisoning (table 1). Apparently schradan is another material which can be safely applied to blooming alfalfa.

Parathion killed a considerably greater number of bees as compared with the other treatments and checks. However, honey production was reduced only slightly. A parathion dust would undoubtedly have caused a greater loss. Where parathion dusts and sprays were compared in field experiments at equal dosages (unpublished data), the former killed twice as many honeybees as the latter. Note the far greater number of bees killed by parathion applied at 3:45 P.M. than at 6:15 A.M., and the high proportion of dead bees collected in front of the hives in the first 24-hour period after treatment (table 1).

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[2] Acknowledgement is due Margaret M. Hard, Elizabeth Donald, and Florence Harris, Home Economics Dept., State College of Washington for conducting honey flavor evaluation tests; Troy Scott, Kermit Groves, and Katherine Day, Dept. of Agricultural Chemistry, State College of Washington, for analyzing honey and alfalfa samples for insecticidal residues.

[3] Carl Johansen is an Assistant Entomologist, and Marvin Coffey a Graduate Assistant, State College of Washington, Pullman; John Quist is now Assistant Entomologist, Colorado A. & M. College, Fort Collins, Colorado.

Table 1.—Effects of insecticide treatments to alfalfa on honey bees and pea aphids.

Treatment[a] and Time of Application	Number Dead Bees in Front of Hives		Average Number Living Aphids/25 Sweeps			Number Frames Honey Produced per Hive in 3 Weeks
	1st 24-hr. Period	2nd 24-hr. Period	Pre-Treatment	1 Day	6 Weeks	
		1954				
demeton—1 qt. 22% per acre—6:30 A.M.	2	6	153	0	1	6.5
demeton—1 qt. 22% per acre—2:45 P.M.	9	5	78	1	2	8
Check	1.5	4	124	108	85	7
		1955				
schradan—1 qt. 42% per acre—6:30 P.M.	8	4	78	28	5	13
schradan—1 qt. 42% per acre—3:30 P.M.	7	5	172	33	3	12
parathion—1 qt. 25% per acre—6:15 A.M.	159	41	71	0	18	10
parathion—1 qt. 25% per acre—3:45 P.M.	1006	113	86	1	34	9
Check	11	4	72	81	58	13

[a] Data for each treatment are based on averages for 2 plots and 4 hives.

All treatments gave good initial control of pea aphids. As expected, the aphid populations in parathion-treated plots were increasing after 6 weeks. Those in demeton or schradan plots remained at a low level.

INSECTICIDE RESIDUES.—Honey samples taken from marked frames (removed 1 week and 3 weeks after treatment) were analyzed for insecticidal residues. A small side-arm suction flask which could be inserted into single honey cells was used to obtain representative samples from each frame. Demeton analyses were based on the cholinesterase inhibition method of Hensel *et al.* (1954); schradan analyses, on the colorimetric method of Hall *et al.* (1951); and parathion analyses, on the colorimetric method of Averell & Norris (1948). Residues on alfalfa samples were also determined.

In the present test, demeton residues in honey were quite small (table 2). Demeton in the nectar of treated plants is very quickly changed to breakdown products, experiments by Thomas & Jones (1955) show.

Table 2.—Insecticide residues detected in honey and alfalfa (p.p.m.).[a]

Treatment and Time	In Honey Produced During		In Alfalfa After	
	Week After Spraying	3 Weeks After Spraying	3 Weeks	6 Weeks
Demeton	0.12	0.12	38.7	0.4
6:30 A.M.	0.14	0.15	77.0	22.8
Demeton	0.12	0.15	15.2	2.1
2:45 P.M.	0.16	0.16	38.6	2.6
Schradan	0.52	0.16	0.42	0.18
6:30 A.M.	0.70	—	0.46	0.20
Schradan	0.00	0.00	0.30	0.00
3:30 P.M.	0.18	0.12	0.38	0.17
Parathion	0.00	0.03	0.04	0.00
6:15 A.M.	0.11	—	0.07	0.02
Parathion	0.00	0.03	0.08	0.05
3:45 P.M.	0.00	0.07	0.46	—

[a] Each analysis is for one composite sample taken from each plot.

Demeton residues found on alfalfa were high and erratic, varying from 0.4 to 77 p.p.m. Data from similar applications on alfalfa in experiments conducted in 1953 yielded an average of 1.0 p.p.m. for 3-week samples and 0.3 p.p.m. for 6-week samples. It has been noted that the cholinesterase method of analysis for demeton has given quite different results when conducted at different laboratories. According to Metcalf *et al.* (1955), demeton residues analyzed by the method used in these tests may be from 5 to 10 times too large.[4] Another factor which may cause discrepancies in residue analyses is the weight basis. All results reported here are based on air dried alfalfa samples. The average loss in weight of these samples from the fresh to the air dried condition was 47% (air dried weight = 53% fresh weight).

Jones & Thomas (1953) found that white mustard nectar contained 5.5 p.p.m. schradan after treatment and 0.3 p.p.m. 1 month later. They also stated that schradan mixed with honey remained quite stable during 2½ months' storage. Schradan residues in honey were somewhat variable in this test. Those analyzed from the sample produced during the first week after the 6:30 A.M. application were definitely higher than the others.

Results obtained from schradan-treated alfalfa samples were quite consistent, with little variation between morning and afternoon applications. They averaged 0.39 p.p.m. after 3 weeks and 0.14 p.p.m. after 6 weeks.

Parathion residues in both alfalfa and honey samples were low. One alfalfa sample from the 3-week period was much higher than the rest and probably represents a technical error in sampling or analysis.

FLAVOR CHANGES IN HONEY.—Honey samples were extracted cold from the marked frames removed 3 weeks after the insecticide applications. Flavor analyses were conducted by a 15-member panel such that each sample was judged 30 times. A triangulation test method was used and each sample was compared with a check sample at each judgment. Honey samples were presented to panel members on plastic discs. No significant differences in flavor were detected in any of the samples from hives in demeton-, schradan-, or parathion-treated plots as compared with check samples.

[4] The principal toxic demeton plant metabolites are about 5 to 10 times more active as cholinesterase inhibitors than technical Systox.

OBSERVATIONS ON HONEY BEE ACTIVITY.—Activity of honey bees was carefully observed for several days following each treatment. In the demeton-treated plots, the bees appeared to be repelled during the first 24 hours. They were "milling around" in an unusual manner, quite agitated, and few were foraging on the alfalfa blooms. A faint odor of demeton was detectable at this time. After 48 hours, the bees appeared to be foraging normally and the demeton odor could hardly be detected, if at all. This activity pattern was consistently observed in all four demeton-treated plots.

In the parathion-treated fields, the bees exhibited abnormal "milling around" and agitation, but quite a few were actively foraging during the first 24 hours. No parathion odor was detected in these fields after 1 day.

No unusual bee activity was observed in the schradan-treated plots after treatment.

CONCLUSIONS.—Honey bees were not adversely affected by demeton or schradan treatments on alfalfa. No greater number of dead bees was collected in front of the hives with these treatments than in the controls. No differences in the amount of honey produced were obtained.

Parathion plots averaged up to 1006 dead bees in front of the hives. There were 6.3 times as many dead bees in the first 24 hours following 3:45 P.M. treatments as there were following 6:15 A.M. treatments. More than 90% of the dead bees recovered in the 48 hours following treatment were collected in the first 24-hour period. Honey production was depressed only slightly in the parathion plots.

Insecticidal residues in honey samples from the treated plots were 0.18 p.p.m. or less with but one exception. The samples produced during the first week following the 6:30 A.M. applications of schradan contained an average of 0.61 p.p.m. of the insecticide. The possible hazard appears negligible under the circumstances, since honey would never be removed at similar intervals, nor would bees be forced to forage one particular field, under normal agricultural practices.

Demeton residues on alfalfa were high and erratic, varying from 0.4 to 77 p.p.m. However, a cholinesterase method of analysis was used, which may account for the results obtained. Schradan residues averaged 0.39 p.p.m. after 3 weeks and 0.14 p.p.m. after 6 weeks. Parathion residues on alfalfa were low, averaging 0.04 p.p.m. except for one sample which yielded 0.46 p.p.m.

No significant differences in flavor were detected in honey samples from the treated plots. Observations indicated that the honey bees were strongly repelled from foraging in the demeton-treated plots during the first 24 hours. Parathion also repelled the bees, but many were foraging the alfalfa blooms during the first 24 hours. Schradan caused no unusual activity by the honey bees. The fact that demeton is absorbed very quickly by plants and also is quite repellent to honey bees may help to explain the apparent lack of bee poisoning hazard in field applications to blooming alfalfa.

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Attempted Transmission of *Pasteurella tularensis* by Three Species of Fleas[1]

DALE D. PARKER, *University of Utah, Dugway*

The presence of *Pasteurella tularensis* in fleas taken from rabbits or rodents in nature has occasionally been reported. These reports, although generally obtained from areas where tularemia reached epizootic prominence among native animals, also appear in non-epizootic areas. The reported incidence of infection in such fleas seems to be exceedingly low in comparison with the thousands of specimens examined and found to be free of the organism.

Naturally-infected fleas were reported in the U.S.S.R. by Volfertz *et al.* (1934) and Olsufiev (1940). Early records in the United States included positives reported in Minnesota from cottontail rabbits and snowshoe rabbits (hares) collected in the summers of 1933, 1934 and 1935 (Green & Shillinger 1933–35). Tularemia-infected fleas from prairie dogs collected in June, 1941, from Carbon County, Wyoming, were reported from the Public Health Service Laboratories at San Francisco, Calif. (Anon. 1941). Fleas, probably of the species *Malaraeus telchinum* Roths. from meadow mice trapped at Alameda County, Calif., were reported by Burroughs *et al.* (1945) to be infected by the organism. In Utah, fleas infected with tularemia were removed from antelope ground squirrels in Tooele County in 1952 (Woodbury & Parker 1954).

These records collectively represent results of large-scale survey programs involving many years of collecting and the examination of large numbers of specimens. Most of the surveys were conducted in epizootic areas where fleas occur on the animals involved in the epizootics. The paucity of the records of infected fleas in such large scale projects has led to the question of why fleas appear to be such poor vectors of the disease.

Prince & McMahon (1946) demonstrated that *P. tularensis* remains viable in dead fleas not longer than 7 days, either dry or in 2% saline storage. When survey specimens are taken in the field and shipped to the laboratory, the loss of time may thus become a critical factor in its detection, and may help to account for the low recorded incidence of the organism in fleas and other ectoparasites.

Fleas have not been shown experimentally to be efficient vectors of the disease. Prince & McMahon (1946) indicated that fleas of two species, *Xenopsylla cheopis* (Rothschild) and *Diamanus montanus* (Baker), readily became infected when they fed on tularemia infected white mice. However, these species failed to transmit the pathogen by biting or feeding on healthy laboratory mice and guinea pigs. No record of fleas transmitting tularemia in nature has been reported.

Interest in fleas and their relationship to tularemia infection of rodents and rabbits in the area of the Great Salt Lake Desert was stimulated, in part, by a record of infected fleas taken from the antelope ground squirrel, *Citellus leucurus leucurus* (Merriam), (Woodbury & Parker 1954), and by the fact that in Utah, cases of tularemia in humans reported annually to the State Department of Health have ranged from 20 to 65 per year during a 17-year period, 1937 to 1953 (Woodbury 1956:85). Although many of the sources of human cases are attributed to contact with animals through trapping and skinning, it is possible that arthropod vectors from diseased animals may be equally significant.

This study was initiated to ascertain experimentally whether some of the more common endemic species of fleas could serve as vectors of *P. tularensis* and thus help to clarify their status in the dissemination of tularemia among native animals and from them to man.

Special acknowledgement is made to Gordon Stagg whose bacteriological and technical guidance during the early phases of this study were invaluable; also to Dr. A. M. Woodbury for support and encouragement. Flea samples of the *Thrassis* group were identified by Harold E. Stark of the San Francisco Field Station of the Communicable Disease Center, U. S. Public Health Service.

MATERIALS AND METHODS.—Fleas of the species *Cediopsylla inaequalis inaequalis* (Baker), *Thrassis bacchi gladiolis* Jordan and *Pulex irritans* L., used in experimental transmission studies were collected from the cottontail rabbits *Sylvilagus nuttallii* ssp. and *S. audubonii* ssp.; the antelope ground squirrel, *Citellus leucurus leucurus* (Merriam) and the desert kit fox, *Vulpes macrotis* Merriam, respectively. After collection, fleas were retained in glass jars having 60-mesh wire screen tops. The bottoms of the jars were covered with a layer of a mixture of moistened plaster of paris and activated charcoal. Experimental fleas were stored at approximately 4° C. for not longer than 5 days.

Pasteurella tularensis (Schu strain) cultures were suspended in a modified casein hydrolysate medium and stored at 4° C. The LD_{50} of the strain was 1 to 10 organisms as determined by the linearized mortality grid method from data obtained 14 days following inoculation (Goldberg *et al.* 1954).

White rats, guinea pigs and antelope ground squirrels intended for use as hosts to the experimental fleas were inoculated with 0.25 ml. (100 to 1000 organisms) dosages of the above suspension of *P. tularensis*. White-footed deer mice. *Peromyscus maniculatus sonoriensis* (LeConte) were inoculated with triturated fleas to determine the presence or absence of *P. tularensis* in experimental fleas. Deer mice were also used for LD_{50} determinations.

Two feeding methods were used in attempts to infect the fleas. In the first method, fleas were placed in small glass attachment tubes, open at both ends. One end of the tube was placed against a shaved area of abdominal skin of the host so the fleas could feed. The other end of the tube was stoppered during the 15 to 20 minutes that the fleas usually required for feeding, after which they were removed from the tubes with an aspirator. Several small groups (5 to 10 fleas) were successively introduced. Those that refused to feed were excluded from the experi-

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ments. In order to restrain the host with abdomen exposed during the flea feeding operation, all four legs were spread and taped to an 18″ by 12″ pine board. The tape was secured on the sides of each leg by driving thumb tacks through the tape into the board. An additional strip of tape was placed across the chest of the animal to prevent side to side rolling motions which usually allowed fleas to escape from beneath the attachment tubes.

In the second method of feeding, fleas were placed in jars with their hosts. By this method 75 to 200 fleas were introduced at one time into 7″ by 7″ glass battery-type jars which contained the infected hosts. A shallow layer of wood shavings and feed (dry grain) covered the bottom of each jar. The open tops of the jars were covered with lids of 60-mesh wire screen held by galvanized metal frames. A $\frac{3}{4}$-inch hole in the frame accommodated a rubber stoppered tube, through which hungry experimental fleas were introduced. After death of an infected host, fleas were removed from the litter in jars by a modified Berlese funneling technique. In this technique, litter containing fleas was placed in a 12″ soil sampler (10-mesh) under an incandescent desk lamp in a white enameled pan. After 1 hour fleas, driven downward through litter and screen by heat and light, were recovered from the bottom of the pan with an aspirator.

To determine if fleas had fed on infected hosts, they were funneled into a glass tube and observed under a dissecting microscope. They were considered to have fed if a distended abdomen with blood (usually dark red in the gut) could be seen.

At autopsy of the carcasses of infected hosts, spleen and liver were removed and streaked on glucose-cysteine-blood-agar (GCBA) plates. Plates were incubated at 37.5° C. for 24 to 36 hours. Rapid slide agglutinations specifically confirmed positive *P. tularensis* cultures.

To prepare fleas for inoculation into deer mice, they were washed in a bath of a quaternary ammonium compound (Roccal) for 2 minutes to disinfect their external parts. The roccal bath was followed by four successive rinses in sterile distilled water. Using Ten Broeck tissue grinders, fleas were ground in 2 ml. of a 2% gelatinized saline solution. Dosages of 0.25 ml. of the triturate per animal were administered subcutaneously at two intervals, (1) immediately after an infected blood meal, and (2) 24 hours after the blood meal. To determine if fleas had previously been infected in nature, 20 freshly caught fleas were tested as controls for each of the experiments. These were sampled for the organism before having been fed on infected hosts.

RESULTS.—*Flea infection experiments, attachment tube feeding method:* In these experiments pools of fleas were fed on tularemia infected host animals at different intervals during incubation of the disease. Part of the fleas were triturated immediately after feeding and were inoculated into healthy deer mice. The remainder, after being held alive 24 hours, were then triturated and inoculated into healthy deer mice. Results of the flea infection experiment using the attachment tube method are presented in table 1. These data show that in two species some individuals became infected by feeding on infected hosts, and retained virulent *P. tularensis* organisms for 24 hours. The data also indicate that only fleas that had fed just prior to the death, or during the terminal bacteremia of the host, became infected.

Transmission experiments, jar feeding method: Three species of fleas were allowed to feed upon infected ground squirrels in jars and later were transferred to clean jars with healthy squirrels. Transmission of *P. tularensis* to healthy antelope ground squirrels was not demonstrated. Samples (5 to 15) were tested from each group of fleas that had fed on infected hosts. Results of transmission experiments using the method of fleas living with their hosts in jars are presented in table 2.

These findings corroborate the results of the first set of experiments which demonstrated that *C. i. inaequalis* and *T. b. gladiolis* could become infected by feeding on infected rodents. The failure of the three species to transmit to new hosts is far from conclusive and further experiments are planned to further test flea potentialities for transmission.

The percentage of infected fleas in each group used in transmission experiments was not recorded. However, in all but two of the experiments, *P. tularensis* infected fleas were found in the small samples. Infected fleas were not present in the two *P. irritans* samples.

Fleas used as controls for all experiments were negative for *P. tularensis*.

DISCUSSION.—It was clearly established that when fleas of the species *C. i. inaequalis* and *T. b. gladiolus* were allowed to feed on infected host animals (rodents), the fleas ingested and retained *P. tularensis* for at least 24 hours. In some of the experiments fleas did not show evi-

Table 1.—*Pasteurella tularensis* infection of fleas in tubes attached to host.

Species	Infected Host	Hours Between Feeding Fleas and Death of Host	Fleas Tested for Organism			
			Immediately After Feeding		24 Hours After Feeding	
			Number	Results	Number	Results
Cediopsylla i. inaequalis	Guinea pig	2	9	Positive	10	Positive
	Antelope ground squirrel	3	9	Positive	9	Positive
	Antelope ground squirrel	5	9	Positive	10	Positive
	Guinea pig	10	16	Negative	16	Negative
	White rat	12–14	15	Negative	0	
Thrassis b. gladiolis	Antelope ground squirrel	1	9	Positive	9	Positive
	Antelope ground squirrel	6	6	Negative	0	
	Guinea pig	7–9	10	Negative	10	Negative

Table 2.—*Pasteurella tularensis* transmission experiments using fleas which were placed in jars with antelope ground squirrel and guinea pig hosts.

Species	Experimental Hosts	Number of Fleas Placed on Hosts		Flea Samples Tested for Organisms	
		Infected	Healthy	Number	Results
Cediopsylla i. inaequalis	Antelope ground squirrel	78	30 30	5	+
		150	50 50	15	+
Thrassis b. gladiolis	Antelope ground squirrel		25 25		
		160	25 25 25	15	+
		190	25 25 25		+
Pulex irritans	Antelope ground squirrel		30 30		
		200	30 30		–
	Guinea pigs		20		
		100	20 20 20	5	–

[a] No transmissions were recorded.
[b] Flea samples were separated indiscriminately from the large groups of fleas under "infected" column, and were triturated and inoculated into healthy deer mice.

dence of having ingested the organisms by feeding on infected hosts. When they did not, it was found that feeding had taken place before bacteremia had had sufficient time to develop in the host. This finding indicates that a "terminal bacteremia" such as that described by Prince & McMahon (1946) for laboratory white mice and guinea pigs, exists in antelope ground squirrels. The term terminal tularemia sepsis in *Microtis* was used by Burroughs *et al.* (1954). Further information of the extent of bacteremia in antelope ground squirrels and other native rodents is needed in order to successfully predict feeding time and also to infect large numbers of experimental ectoparasites on a given host.

The failure to detect *P. tularensis* in *P. irritans* cannot be explained by these data. However, the fact that the small (5 fleas) samples were not infected does not exclude the possibility that some of the larger groups (80 to 120) of this species might have been infected.

Experiments designed to determine retention of *P. tularensis* by fleas for periods greater than 24 hours were restricted because of the difficulty of maintaining them in sufficient numbers long enough to continue the studies. Little success was had in rearing these species in the laboratory and regardless of precautions exercised in holding techniques, vitality of fleas decreased to the extent that it was impossible to prolong persistence studies.

Upon examination of fleas up to 7 days following a meal of infected blood, no evidence of blockage in the digestive system was observed. Generally the entire gut became cleared of the bulk of a blood meal within 36 hours after feeding on tularemia infected animals. Whether or not virulence or viability of the organisms remains constant within the flea is not known, but further investigation is in progress to determine if a bactericidal or bacteriostatic factor might be involved.

It was evident in these experiments that no transmission of *P. tularensis* was accomplished by any of the fleas under the experiment. Repeated biting and feeding on healthy hosts by infectious fleas was observed in all transmission trials. Although these trials furnished evidence of low vector efficiency of these species under experimental conditions, there is no evidence that *P. tularensis* can not be transmitted from host to host in nature.

SUMMARY.—Fleas of the species *Cediopsylla i. inaequalis* and *Thrassis bacchi gladiolis* ingested *P. tularensis* organisms when allowed to feed on moribund tularemia infected guinea pigs and antelope ground squirrels. This occurred both when fleas had fed in a tube with the open end applied to the shaved abdomen of the host and when they had been allowed to live in jars with infected hosts.

C. i. inaequalis and *T. b. gladiolis* retained *P. tularensis* organisms for at least 24 hours.

Twenty separate trials using groups of from 20 to 50 fleas per trial failed to transmit tularemia from infected hosts to healthy hosts.

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Transmission of Bean Yellow Mosaic Virus by Aphids[1]

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The bean yellow mosaic virus causes a serious disease of beans and is widely present in certain clovers and in gladiolus. The investigations reported herein were started primarily because of the prevalence of this virus in gladiolus.

Aphid Species Capable of Transmitting Bean Yellow Mosaic Virus.—Aphid-transmitted viruses vary in number of vectors from the citrus quick-decline virus which is transmitted very inefficiently by a single aphid species (Dickson *et al.* 1956) to the onion yellow dwarf virus which can be transmitted by many aphid species (Drake *et al.* 1933). The bean yellow mosaic virus has been transmitted experimentally by *Aphis craccae* L. (Heinze 1952), *A. fabae* Scop. (Crumb & McWhorter 1948), *Macrosiphum pisi* (Harris) (Hagedorn & Walker 1950, Pierce 1934, Swenson 1954). *M. solanifolii* (Ashm.) (Pierce 1934, Swenson 1954) and *Myzus persicae* (Sulz.) (Frandsen 1952, Swenson 1954). *Brevicoryne brassicae* (L.) failed to transmit this virus (Frandsen 1952, Swenson 1954). This information is not enough to demonstrate whether the virus could be transmitted by few or many species. Consequently, a number of other aphid species were tested for their ability to transmit the bean yellow mosaic virus.

Methods.—In most of these tests, the aphids were on the diseased plant for 15 to 60 minutes. In some cases (*Amphorophora* sp., *Aphis cardui*, *A. fabae*, *A. gossypii* and *Macrosiphum rosae*), the period on the diseased plant was of several hours duration. After feeding on the diseased plant, the aphids were transferred to healthy indicator plants at the rate of 20 aphids per plant. The feeding period on the healthy plants was 1 hour or more. In most tests, broad bean (*Vicia faba* L.) and Dwarf Horticultural bean (*Phaseolus vulgaris* L.) were used both as virus source plants and as healthy indicator plants. Alaska pea (*Pisum sativum* L.), alsike clover (*Trifolium hybridum* L.), and crimson clover (*T. incarnatum* L.) also were sometimes included. The strain of bean yellow mosaic used in all these tests was that described by Swenson (1954).

Results.—Table 1 shows the results obtained with 17 species of aphids. Sixteen of the 17 species transmitted the virus. Fourteen of these species had not previously been reported as capable of transmitting the bean yellow mosaic virus. These 14 species bring to a total of 19 the number of aphid species for which transmission of bean yellow mosaic virus has been demonstrated.

The only species which did not transmit the virus in these tests was *Brevicoryne brassicae*. Previous tests with this aphid had also given negative results (Frandsen 1952, Swenson 1954). It was tested again, however, after so many other species had transmitted the virus. No transmission was obtained with 800 individuals of this species. Although Frandsen (1952) reported negative results with *B. brassicae*, he stated that his tests should not be regarded as conclusive since conditions were not optimal for transmission. Neither, however, were conditions of the tests in which he obtained transmission with *Myzus persicae*, nor conditions for the 16 species that transmitted the virus in the tests reported herein (table 1).

These tests show that the bean yellow mosaic virus can undoubtedly be transmitted by many aphid species under field conditions. It is similar in this respect to the common bean mosaic (Zaumeyer & Kearns 1936), western celery mosaic (Severin & Freitag 1938) and onion yellow dwarf viruses (Drake *et al.* 1933).

Discussion.—With the exception of potato viruses (Broadbent 1953), the ecology[2] of the aphid-borne plant viruses is probably the most neglected aspect of plant virus study. The bean yellow mosaic virus is no exception in this respect. Information acquired by various workers for other reasons permits, however, a generalized conception of the insect-plant interrelationships responsible for the maintenance of this virus in the field. The purpose of the subsequent discussion is to point out the significance of the capacity of many aphid species to transmit the bean yellow mosaic virus in certain crops

The host range of bean yellow mosaic virus has been reviewed recently by Frandsen (1952) and by Goodchild (1956). The strains or isolates used by different workers have varied somewhat but, in general, bean (*Phaseolus vulgaris* L.), broadbean, vetch (*Vicia sativa* L.), sweetpea (*Lathyrus odoratus* L.), white sweetclover (*Melilotus*

Table 1.—Bean yellow mosaic virus transmission tests with 17 aphid species.

Aphid	Number of Plants	
	Used[a]	Infected
Amphorophora sonchi (Oestlund)	15	3
Amphorophora sp.[b]	7	2
Anuraphis tulipae (Fonsc.)	20	7
Aphis cardui L.	20	5
A. fabae Scop.	14	10
A. gossypii Glover	10	8
A. helianthi Mon.[c]	20	7
Brevicoryne brassicae (L.)	40	0
Capitophorus fragaefolii (Ckll.)	17	3
Hyalopterus atriplicis (L.)	40	1
Macrosiphum barri Essig	12	6
M. granarium (Kby.)	20	4
M. rosae (L.)	15	13
M. solanifolii (Ashm.)	20	7
Myzus cerasi (Fabr.)	10	2
M. certus (Wlk.)	20	16
M. solani (Kltb.)	20	3

[a] 20 aphids per plant.
[b] From *Rubus*.
[c] This species was determined as *Aphis helianthi* Mon. by Miss Louise Russell. The writer is also indebted to Miss Russell for determination of *Anuraphis tulipae*, *Macrosiphum granarium*, *M. solanifolii* and *Myzus certus*.

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[2] The concept "ecology of viruses" might be interpreted in two ways. One way is to consider the ecology of the virus particles themselves. For plant viruses, the environment involved in such a consideration would be primarily that within the host or vector. Exceptions would include soil-borne viruses. The alternative interpretation would involve the ecology of the host plants and vectors of the viruses, since these are responsible for the maintenance of the virus in nature. The factors affecting the abundance and distribution of the hosts and vectors would also thus determine the prevalence of the virus. Consideration of the ecology of viruses in terms of the hosts and vectors is the usage followed by Burnet (1955) and Shope (1954).

albus L.), yellow sweetclover (*M. officinalis* (L.) Medik.), some pea varieties, alsike clover and crimson clover have been infected. Results with red clover (*Trifolium pratense* L.) and soybean (*Glycine max* Merr.) have been quite variable. Gladiolus was found susceptible to bean yellow mosaic virus (Berkeley 1953, Bridgmon & Walker 1952, McWhorter *et al.* 1947) and, more recently, Freesia and onion (Van Slogteren 1952). Most workers have reported negative results from attempts to transmit bean yellow mosaic virus to alfalfa. McWhorter (1949) has, however, recovered this virus from naturally-infected alfalfa, indicating that some strains will infect alfalfa.

Clover and alfalfa are the principal reservior of the bean yellow mosaic virus. The virus is carried from these to other susceptible crops by the pea aphid, (*Macrosiphum pisi*). Virus spread within pea, alfalfa and clover plantings can also be attributed to the pea aphid. No pea aphid populations develop on beans and gladiolus, however.

Zaumeyer & Kearns (1936) found the alates of 14 aphid species on beans early in the season where they had alighted during migration. Their transmission tests showed 11 species capable of transmitting the common bean mosaic virus. They concluded that the spread of this virus is the result of transmission by alate aphids migrating through the bean fields where these aphids acquired the virus from diseased plants and transmitted it to healthy plants. They also concluded that the particular aphid species involved would depend on the plants or crops growing in the vicinity of the bean fields. These conclusions appear also to apply to the spread of the bean yellow mosaic virus in beans, with one difference. Common bean mosaic virus can be introduced into a field by seed transmission but this does not appear to occur with the bean yellow mosaic virus (Frandsen 1952, McWhorter *et al.* 1947, Pierce 1934). Consequently, initial introduction into bean fields, as well as subsequent spread, is dependent upon aphids. Observations indicate that the role of aphids in bean yellow mosaic virus spread in gladiolus is similar to that in bean. Alate aphids can be found on gladiolus through most of the growing season. Introduction of the virus into the gladiolus field does not depend on aphids since the virus can be carried in the corms. The fact that numerous aphid species are capable of transmitting the virus indicates that many, if not most, of the aphids occurring on gladiolus or beans will be potential vectors.

Heinze (1952) has divided aphid vectors of virus diseases into two groups: normal vectors and occasional vectors (*Gelegensheitüberträger*). Normal vectors breed on the crops to which they transmit viruses; occasional vectors do not. Thus for normal vectors the plant can be a host for both the virus and the aphid. The transmission of bean yellow mosaic virus to gladiolus and beans appears to depend primarily upon aphid species which do not breed on these plants. The same is true of the transmission of common bean mosaic (Zaumeyer & Kearns 1936), of melon mosaics in southern California (Dickson *et al.* 1949) and of onion yellow drawf (Drake *et al.* 1933). For a proper conception of the spread of aphid-borne viruses, it is necessary to understand that in some crops certain viruses are normally transmitted primarily by aphid species which do not reproduce on these plants. Consequently, Heinze's classification is misleading.

EFFICIENCY OF TRANSMISSION.—To obtain information on the efficiency of transmission, a series of replicated tests was made under conditions more favorable for transmission than the conditions of the initial test (table 1). Five aphid species were included. *Amphorophora sonchi* (Oestlund), *Brevicoryne brassicae*, *Macrosiphum pisi*, *Myzus persicae*, and *Anuraphis tulipae* (Fonsc.). *Myzus persicae* was included in each replicate but the other four were not. Consequently, in summarizing (table 2), transmission by each species is compared with that by *M. persicae* in the replicates in which both were included.

The aphids were starved for 4 to 6 hours before feeding on the diseased plant. They were then placed on virus-infected broadbean and watched until the first feeding terminated naturally. Actually, transmission tests are directly comparable only when the aphids feed for the same length of time since efficiency of transmission of non-persistent viruses varies with the length of the feeding period on the diseased plant. The use of naturally-terminated feeding periods may not fulfill this requirement. It is, however, likely to give a better estimate of relative efficiency of the species under natural conditions and to provide information on the feeding behavior of the aphids. After the aphids fed on the diseased plant, they were transferred, one per plant, to healthy broadbean. Ten healthy plants were included in each replicate for each aphid species. The aphids remained on the healthy plants for at least 1 hour before removal by nicotine fumigation.

Myzus persicae was most efficient, followed by *Macrosiphum pisi* and *Anuraphis tulipae* (table 2). *Amphorophora sonchi* was very inefficient. No transmission was obtained with *Brevicoryne brassicae*. This species was included to prove that the negative results in previous tests were not due to its failure to feed. Table 3 shows the cumulative percentages of aphids which fed for different time intervals. The differences in transmission between the species are not likely to be explained on the basis of feeding habits.

LOSS OF APHID TRANSMISSIBILITY BY AN ISOLATE OF BEAN YELLOW MOSAIC VIRUS.—Experiments were started in late 1955 to determine the possibility of dif-

Table 2.—Comparison of transmission efficiency of bean yellow mosaic virus by five aphid species.

SPECIES	PER CENT TRANSMISSIONS	NUMBER OF PLANTS[a]
Amphorophora sonchi	1	100
Myzus persicae	25	100
Anuraphis tulipae	10	50
Myzus persicae	28	50
Brevicoryne brassicae	0	50
Myzus persicae	20	50
Macrosiphum pisi	16	50
Myzus persicae	24	50

[a] One aphid per plant.

Table 3.—Feeding behavior of five aphid species on broadbean infected with bean yellow mosaic virus.

	Cumulative Per Cent Aphids Which Completed Feeding by End of (Seconds)						
	10[a]	20	30	40	50	60	120
Amphorophora sonchi	0	4	28	49	58	67	92
Anuraphis tulipae	2	12	32	54	64	70	88
Brevicoryne brassicae	2	8	30	38	42	46	64
Macrosiphum pisi	0	28	56	70	80	84	94
Myzus persicae	0	17	61	75	89	92	96

[a] This category includes those aphids which fed for 10 seconds. The very few which fed less than 10 seconds were discarded.

ferential susceptibility between bean varieties to aphid inoculation with bean yellow mosaic virus. An unexpectedly low rate of transmission occurred in these tests. Successive transmission tests indicated that a marked reduction in efficiency had occurred. The following section describes a series of tests which resulted in the conclusion that this isolate had lost its aphid transmissibility.

MATERIALS.—The virus used in these these tests was obtained from red clover near Geneva, N.Y., in 1953. It had been maintained since early 1954 in two lines subinoculated from a single alsike clover plant inoculated in the initial experiments (Swenson 1954). One of these lines, herein designated Isolate I, had been maintained by the writer and the other, designated Isolate II, by F. P. McWhorter. Isolate I was maintained in broadbean by mechanical inoculation from early 1954 until the summer of 1955, at which time it was used in aphid transmission tests and was transmitted readily by several aphid species. Isolate II had been maintained in broadbean by mechanical inoculation since early 1954.

RESULTS.—Series I. These tests were started in late 1955. Pea aphids, were placed for 5 to 10 minutes on broadbean inoculated mechanically with Isolate I. The aphids were then transferred to healthy bean plants at the rate of five aphids per plant. After 1 to 2 hours on the healthy plants, the aphids were removed by fumigation with nicotine. Five varieties of beans were included: Dwarf Horticultural, Blue Lake (OSC 22), OSC 14–12 Red Kidney and Bountiful. The test was replicated five times. Each replicate included 10 plants of each of the five varieties. Eight of the 250 bean plants were infected.

Some workers have experienced difficulty in getting good symptoms of bean yellow mosaic virus in beans during winter months. The results of a series of mechanical inoculations indicated that the low rate of aphid transmission was not explainable in this way. Five replicates of 10 plants of each of the five varieties were inoculated mechanically. One hundred eighteen of the 250 plants were infected.

Series II. In these tests, pea aphids were starved for 1 to 2 hours before transfer to the diseased plants where they fed for 10 to 60 seconds. The diseased plants had been inoculated mechanically with Isolate I. After feeding on the diseased plants, the aphids were transferred, one per plant, to 10 plants of each of two bean varieties, Bountiful and Dwarf Horticultural, and left there for at least 2 hours before removal by nicotine fumigation. This test was replicated 10 times. Beans were used as virus source plants in five replicates, peas as source plants in three replicates, and broadbeans in two replicates.

A similar test was made with the green peach aphid. The aphids were handled in the same manner and the same two bean varieties were used as healthy indicator plants. This experiment was replicated nine times. Broadbeans were used as virus source plants in six replicates and peas in three replicates.

One of the 200 bean plants was infected by the pea aphid and one of the 180 plants was infected by the green peach aphid. The preceding statement requires some qualification. These two infected plants showed an aberrant type of symptom which began with a yellowish cast on the first leaf showing symptoms and appeared subsequently as a very mild mottle. There was no marked stunting or conspicuous mottle such as usually developed after inoculation with Isolate I. These two plants were used as inoculum for mechanical transfers to seven Dwarf Horticultural beans. No transmission occurred. Eight plants were inoculated mechanically with Isolate I in a parallel series and seven plants were infected. Various interpretations of these results are possible. Observation of uninoculated healthy bean plants over a period of years indicates, however, that the appearance of these two plants was the result of virus infection.

Series III. At this point, Isolate II was obtained from F. P. McWhorter and was readily transmitted by the green peach aphid. Transmission of Isolate I and Isolate II by three aphid species was compared. The aphid species were the melon aphid, (*Aphis gossypii* Glover), the green peach aphid and the pea aphid. Two colonies of the green peach aphid, obtained from different sources, were used in these tests.

The aphids were placed on the diseased plants for 15 to 60 minutes, after which they were transferred to healthy plants where they remained for 1 hour or more before removal by nicotine fumigation. Twenty aphids of each species or colony were transferred to each of 20 plants. The results are presented in table 4.

None of the aphids transmitted Isolate I whereas all three species transmitted Isolate II. Since the rate of transmission of Isolate II was not high, it is possible that Isolate I might have been transmitted if more aphids had been used. Experience has indicated, however, that an aphid species will usually transmit a non-persistent virus in a test of this type and scope, if it is capable of doing so. The rate of transmission obtained with Isolate II is typical for a test of this type.

Series IV. Transmission of the two isolates by the green peach aphid was compared. The aphids were starved 15 to 45 minutes and then fed on diseased broadbean plants for 15 to 60 seconds. They were then transferred, one per plant, to healthy plants where they remained for at least 1 hour before removal by nicotine fumigation. The experiment was replicated 10 times.

Each replicate consisted of 10 healthy plants to which single aphids that had fed on plants infected with Isolate I had been transferred and 10 healthy plants to which single aphids that had fed on plants infected with Isolate II had been transferred. Broadbean was used as an indicator plant in 12 replicates and Dwarf Horticultural bean in eight replicates. Thirty-four of 100 plants were infected with Isolate II and none of 100 with Isolate I.

Effect of dilution.—Consistent small differences between

Table 4.—Transmission of Isolate I and Isolate II by three aphid species, using 20 aphids per plant, following a 15 to 60-minute acquisition feeding period.

	Isolate I			Isolate II		
	Source Plant	Indicator Plant	Results[a]	Source Plant	Indicator Plant	Results[a]
Aphis gossypii	Bean	Bean	0/20	Bean	Bean	4/20
Myzus persicae						
Colony I	Broadbean	Bean	0/20	Bean	Bean	10/20
Colony II	Broadbean	Bean	0/20	Broadbean	Bean	6/20
Macrosiphum pisi	Broadbean	Broadbean	0/20	Broadbean	Broadbean	3/20

[a] Denominator equal number plants inoculated; numerator equal number infected.

the two isolates were obtained when juice from mechanically-inoculated Dwarf Horticultural beans was diluted over a range from 1:10 to 1:5000. Isolate I consistently infected about 10 to 20% more bean plants at each dilution that Isolate II.

Symptoms on infected plants.—After all the aphid transmission tests had been completed, both isolates were inoculated mechanically into certain common hosts of the bean yellow mosaic virus. The results were within the range of symptom expression expected with this virus. Isolate I produced more severe symptoms in Dwarf Horticultural beans than Isolate II when the plants were inoculated in the primary leaf stage. Symptoms appeared about 2 days earlier on alsike clover inoculated with Isolate I than with Isolate II.

DISCUSSION.—The results show that Isolate I has not been transmitted by any of the last 2,030 aphids in tests involving the use of 130 plants. It appears to have lost its aphid transmissibility, although there is always the possibility that transmission might take place if a sufficiently large number of aphids were used.

There are previous reports of the loss of insect transmissibility by plant viruses. Black (1953) reported the loss of leafhopper transmissibility by three isolates of potato yellow dwarf virus. These isolate were maintained in plants for 12 to 16½ years without insect transmission. At the end of this time, they were no longer transmissible by leafhoppers. He interpreted these results as indicating a mutation to a form not transmissible by leafhoppers which then became dominant in the absence of leafhopper transmission. Hollings (1955) reported loss of aphid transmissibility by chrysanthemum aspermy virus after 2 years of sap inoculation through tobacco plants. This may also be explained as Black has explained the loss of insect transmissibility by potato yellow dwarf virus. Watson (1956) found that potato virus C could be maintained in tobacco by aphid transmission but not in potato.

There remains the problem of trying to account for the loss of aphid transmissibility by Isolate I. Certainly the virus, not the vector, changed as indicated by the failure of three species to transmit it. Also, the loss of transmission was abrupt. Only 1 to 4 months before the decreased rate of transmission was noticed, several aphid species had transmitted it readily. Subsequent to these tests, source plants for aphid transmission tests were inoculated mechanically. After the first indications of reduced transmission (Series I, above), source plants in all tests were inoculated mechanically in an effort to establish the reason for the low rate of transmission. Mutation is a possible explanation for this reduced rate of aphid transmission. If a mutation had occurred resulting in a virus type not transmissible by aphids and this form had multipled to the virtual exclusion of aphid transmissible forms, it would have been preserved by subsequent mechanical transmission. The loss of insect transmissiblity by this bean yellow mosaic virus differs from that reported for potato yellow dwarf virus (Black 1953) and for chrysanthemum aspermy virus (Hollings 1955) in that this virus lost its aphid transmissiblity in a relatively short time. The loss of transmission in all three cases could, however, be explained by mutations.

SUMMARY.—The bean yellow mosaic virus was transmitted by 16 aphid species. Only one species tested failed to transmit the virus. The significance of the low degree of vector specificity in relation to the spread of the virus in beans and gladioli is discussed.

Of four species tested for transmission efficiency, *Myzus persicae* (Sulz.) was most efficient. In decreasing order of efficiency were *Macrosiphum pisi* (Harris), *Anuraphis tulipae* (Fonsc.) and *Amphorophora sonchi* (Oestlund).

An unexpectedly low rate of aphid transmission was enountered with an isolate of bean yellow mosaic virus. No transmission was obtained by the last 2,030 aphids used, athough three aphid species and different combinations of virus source and indicator plants were used. The results indicate a loss of aphid transmissibility for this bean yellow mosaic virus.

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Resistance to American Foulbrood in Honey Bees. II. Differential Protection of Larvae by Adults of Different Genetic Lines[1]

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Exceptional response to the disease American foulbrood by an occasional honey bee colony was reported by several reliable observers prior to 1930 (reviewed by Thompson 1955). To investigate the question presented by these sporadic reports, Park, Pellett, and Paddock obtained and tested a number of colonies which had been reported as exhibiting resistance to American foulbrood. From this experiment they reported (Park 1936, 1937) that resistance to American foulbrood did exist. Later, from additional work, they concluded (Park *et al.* 1937) that not only did resistance to American foulbrood exist in honey bees, but that it was heritable.

At the time of Park, Pellett and Paddock's work, the causative organism (*Bacillus larvae* White) of American foulbrood was known and much of its life history had been described (White 1920). Tarr concluded in 1937 that only the spore stage of the organism would infect healthy brood. The period of host susceptibility was reported by Woodrow (1941a, 1942) and Katznelson & Jamieson (1950) to correspond closely with the mass feeding period. Woodrow concluded that the brood was most susceptible during the first day of larval life, and was no longer susceptible "2 days and 5 hours after hatching." The spores upon being ingested by larvae of susceptible age germinate within a day (Woodrow & Holst 1942). But according to Holst (1946), even though vegetative rods are present in this early phase, the larvae seem to suffer no ill effects, nor are the adult bees able to detect such diseased individuals at such an early stage. From the third day of larval life until 3 days after the cell is sealed the number of bacteria even decreases. Then, a period of multiplication of the rods begins which seems to be associated with the time of larval tissue adjustment preparatory to pupation. White (1920) observed that death of the host occurred during the last 2 days preceding pupation or the first 2 days of the pupal period.

The minimum infective dose for a colony of bees was observed by Sturtevant (1932) to be 50 million spores in 1 liter of 50% sugar sirup, while Woodrow (1942) concluded that a single spore apparently was sufficient to cause the disease in a larva.

Varying degrees of resistance to American foulbrood exist in honey bees. The nature of this resistance, however, is only slightly understood. Resistance has been thought to be primarily an adult hygienic behavior characteristic. The adults are able to detect and remove diseased brood before the pathogen reaches the infectious spore stage (Woodrow 1941b, 1942, Woodrow & Holst 1942, and Woodrow & States 1943).

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[2] The data reported here formed part of a thesis submitted by Victor C. Thompson in partial fulfillment of the requirements for the degree Master of Science.

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Certain of Park's unpublished observations about the mid-1940's indicated the inadequacy of hygienic behavior as the total explanation of resistance. Eventually Rothenbuhler & Thompson (1956) presented clear evidence for innate resistance in larvae, and presented a new framework of ideas which was guiding their research on the problem of resistance to American foulbrood in bees.

This paper is concerned primarily with results which were obtained in 1953. The experiment was designed to determine whether adult bees of a resistant line could inhibit the initial development of American foulbrood in a colony of bees fed an infectious concentration of *Bacillus larvae* spores.

MATERIALS AND METHODS.—Four, two-story, experimental colonies were established, two with adult bees from a resistant line and two with adult bees from a susceptible line. One colony with adult bees from the resistant line and one colony with adult bees from the susceptible line were fed sirup containing spores, whereas the remaining two colonies were fed sirup containing no spores (check treatment).

Two lines of Italian bees were used and are designated as resistant and susceptible. The resistant line was started from two mated queens, M 384 and M 385 (M stands for mating), secured from Hawaii in 1951. These two matings came from stock with a history of resistance in Hawaii. Queens of the resistant line used in this study were representatives of the second generation stemming from the initial two queens. The susceptible-line queens were representatives of the first generation reared from a very susceptible mating (M 331) obtained from a commercial beekeeper in New York. All matings made in our laboratory were by artificial insemination. No claim is made that these are homozygous lines, but they definitely have a widely separated origin and were different genotypically and phenotypically. This experiment sought to determine whether they would show a different response to a pathogen administered in a given way. Each line furnished both sealed brood (to provide adult bees) and eggs (to provide test larvae) to the experimental colonies. All queens used within each line were sisters.

Since the individual matings had not been previously tested for American foulbrood resistance, it was considered desirable to compare lines rather than matings. Thus, the experimental nurse colonies were established and maintained as follows: Each of four colonies was made up of 10 frames with brood and an amount of bees that had covered approximately 15 frames. Two were established from nine colonies in the resistant line and two from 11 colonies in the susceptible line. A mated commercial queen was introduced to each colony, but she was confined to the first (bottom) story. Her brood, however, was removed periodically before her progeny could interfere with the test. Each colony was maintained at adequate strength throughout the study by the addition, at 4-day intervals, of two combs with sealed brood selected from the appropriate supply colonies in the resistant or susceptible lines. Selection of brood and/or bees for the make-up and maintenance of experimental colonies was made in as nearly a random manner as was considered possible.

When the combs of "nurse" brood (to supply adult bees upon their emergence) were added to a colony, they were placed near the center of the second story. As more combs were added, the ones added earlier were moved toward the sides. After the brood emerged the combs were removed.

Although different levels of resistance in larvae of resistant and susceptible colonies had not yet been shown to exist, it was considered desirable to use eggs from both of the lines in the event that larval resistance might be present in one of the lines under study. (Since this investigation, different levels of larval resistance in different genetic lines have been reported by Rothenbuhler & Thompson in 1956.) The eggs upon hatching became the larvae which the nurse bees in the experimental colonies cared for. A comb of eggs was secured by caging a queen on a suitable comb, for a period of 2 days, by means of a zinc-excluder, individual-comb cage. At the end of this period the queen was removed and the bees were brushed gently from the comb. It was then placed between frames of emerging "nurse" brood in the second story of an experimental nurse colony. Two such combs of eggs, one from a queen of the resistant line and one from a queen of the susceptible line, were placed in each nurse colony at 4-day intervals.

As more combs of eggs were added to a nurse colony, the older experimental brood was moved toward the sides in the same manner as was the "nurse" brood. The experimental brood was removed to a fifth colony on the 14th day after introduction to lessen the possibility of newly introduced larvae from becoming infected from any remains of foulbrood-infected larvae that were present. This fifth colony cared for all of the brood from the four experimental colonies until the final count was made. Twenty combs of larvae, 10 from each line, were cared for by each experimental colony during the course of the experiment, making a total of 80 combs studied in the four experimental colonies.

It was impossible for any queen to supply eggs to all four experimental colonies simultaneously. Thus, an orderly scheme of egg-supply was inaugurated once a queen was selected at random to begin furnishing eggs to the experimental colonies. First, she supplied eggs to a spore-fed colony, then 2 days later, she furnished eggs to a check colony. After an interval of a few days she supplied eggs to the other two colonies in like manner. This interval was to permit her to replenish her own colony with eggs since she also was supplying "nurse" brood to the experimental colonies.

Each experimental nurse colony in the spore treatment received approximately 500 million spores in 1 quart of 50% sugar sirup each day for the first 20-day period and approximately 5 billion spores per day during the last 20-day period. These concentrations were 10 times and 100 times, respectively, the minimum concentration reported necessary to cause infection (Sturtevant 1932). Originally only one concentration was planned. But soon after the experiment started, it was evident that the number dying with American foulbrood was very low, thus it was considered desirable to increase the concentration of spores during part of the study. The controls (check treatment) received sugar sirup without spores during the entire experiment. The spores were furnished by Dr. A. P. Sturtevant and the above spore concentrations were based on information which he supplied. Feed-

ing was by means of division-board feeders in the top story; and even though a honeyflow was in progress part of the time the bees nearly always emptied the feeders daily.

Five observations were made on each experimental comb of brood. The first count was made at the time the comb with eggs was transferred to an experimental nurse colony, the eggs then being up to 2 days old. Additional observations were made on the cells containing the individuals hatching from these eggs. These were made 4, 9, 14 and 18 days following the egg count. At each observation the cells were examined for healthy, dead, or missing individuals. Since field diagnostic methods alone were used to classify the dead, only those individuals displaying the typical "ropy" stage were recorded as dying of American foulbrood (AFB). All other dead were listed as unknown dead. At the last observation all cells were uncapped, if sealed, and the final count was made.

To insure observation of the same groups of cells at each count a cardboard template was constructed so that five rows of approximately 30 cells each were visible in each of three areas for each side of a comb. Usually there was sufficient brood so that only two rows of cells needed to be observed in each of the six areas of a comb.

The experiment began June 1, 1953 with 11 mated queens in the susceptible line, and nine mated queens in the resistant line. By the time it ended, July 31, several queens had been lost due, at least in part, to the extreme amount of handling they received. Fortunately most of the loss occurred during the latter part of the study.

From the beginning of the experiment until its end, there probably was a gradual build up of infectious stores in each spore-fed colony because the sirup which a colony did not use immediately was stored. Hence the quantity of infectious material in a colony probably was never constant but was always increasing. Because of these changes the data have been analyzed from the standpoint of the total counts only. Chi-square comparisons were made on such totals, with the values of chi-square being computed by the Brandt and Snedecor method outlined in Fisher (1932).

By use of a good coloring agent added to the sirup it was observed that spore-contaminated sirup was deposited throughout the brood nest and eventually throughout the hive in the case of each colony. By changing colors periodically it was noted that the bees moved much of the sirup from its original storage cells to other areas in the colonies so that many cells may have been contaminated with spores. In spite of the fact that a honeyflow was in progress during much of the study most of the nectar- and honey-storage cells were tinted with the colored sirup. These observations are in general agreement with some reported by Park (1925). With contaminated sirup present throughout the spore-fed colonies, it would seem that the probability of every larva being fed by nurse bees in close contact with spores was high.

RESULTS AND DISCUSSION.—The data collected from the first part of this study are presented in table 1. The first column shows which mating furnished the eggs that were put into the experimental colonies. The queen involved in a given mating number was confined on an experimental comb for 2 days. At the end of this time the eggs which she had laid in the experimental areas were counted. The first larval count (base count) was made 4 days later. Three more counts, 9, 14, and 18 days from the egg count, were made to determine the number of individuals dead of American foulbrood (AFB), dead of unknown causes (unknown dead), and missing. The number of healthy individuals at the final count is given in the last column.

There was considerable loss between the egg count and the first larval count in certain instances. This was not unexpected since the matings involved were between more or less closely related individuals. Inbreeding has been reported to lower egg viability (Mackensen 1951, Cale 1952).

The important consideration, however, is whether any of the loss observed was due to treatment given, that is, spore feeding versus no spore feeding. From the review of the facts known regarding the development of American foulbrood in the host no effect due to treatment would be expected. A total of 2954 eggs (21.5%) was lost in the spore treatment compared to 3000 (21.1%) lost in the check treatment. A chi-square comparison gives a value of 1.268, d.f. = 1, P = between 0.30–0.20. These results, indicating no difference in egg loss between treatments, are as expected.

Since no difference in egg loss due to treatment was evident the first larval count was selected as the base count from which to calculate the percentages and to make the chi-square comparisons of the dead, the missing, and the healthy individuals.

In table 2, the data from table 1 are summarized and analyzed. Total numbers, percentages, and chi-square comparisons are given by nurse bee groups within treatments. Comparisons have been made on the total numbers only. This was done, as stated previously, because of the changing amount of infectious material in a colony. It is seen in table 2 that the effect of spores increased the percentage of dead (AFB and unknown dead) and missing, and decreased the percentage of healthy pupae.

Within the spore treatment the number of individuals observed dead of American foulbrood in the samples of brood nursed by adults from the resistant line was 18 (0.33%) and in the samples of brood nursed by the susceptible line was 122 (2.28%), giving a chi-square of 79.972 with P = less than 0.001. The 18 individuals observed dead of American foulbrood in the samples of brood cared for by nurses from the resistant line were recorded from only six of the 20 combs under study, whereas the 122 individuals observed dead of American foulbrood in the samples cared for by nurses from the susceptible line were recorded from 17 of the 20 combs under study. It is of interest to consider these results in terms of what would be expected if the above 40 combs represented 40 colonies—20 of resistant stock and 20 of susceptible stock, and all of them were fed infectious sirup of the same dosage as was used in this investigation. In the case of the resistant stock six colonies would be expected to develop American foulbrood, whereas in the case of the susceptible stock 17 colonies would be expected to develop it. A similar comparison could be made, comparing the 40 combs in the experiment discussed herein to a commercial apiary of 20 resistant colonies and 20 susceptible colonies. If all of these colonies engaged in robbing from some other colony which was heavily

Table 1.—Individuals recorded as healthy, dead, and missing in sampled areas of experimental combs.

Nursed by Adults from Resistant Line							Nursed by Adults from Susceptible Line						
Source of Eggs[a]	Date of Base Count	Base Count (2nd Count)	Observed Dead (3rd through 5th Count) AFB	Observed Dead (3rd through 5th Count) Unknown	Missing	Healthy Pupae (5th Count)	Source of Eggs[a]	Date of Base Count	Base Count (2nd Count)	Observed Dead (3rd through 5th Count) AFB	Observed Dead (3rd through 5th Count) Unknown	Missing	Healthy Pupae (5th Count)
Spore treatment													
M 519	6-9	117	0	1	8	108	M 532	6-9	257	0	2	19	236
M 460	6-9	272	1	2	13	256	M 467	6-9	212	0	3	15	194
M 534	6-13	337	0	3	26	308	M 535	6-13	314	6	2	10	296
M 455	6-13	314	0	2	14	298	M 458	6-13	246	0	3	9	234
M 532	6-17	275	0	10	36	229	M 519	6-17	177	2	0	9	166
M 505	6-17	260	1	8	6	245	M 460	6-17	278	2	0	4	272
M 535	6-21	307	0	1	10	296	M 534	6-21	277	1	1	5	270
M 458	6-21	186	0	0	8	178	M 455	6-21	297	1	1	9	286
M 519	6-25	251	0	0	12	239	M 531	6-25	258	1	0	11	246
M 504	6-25	248	0	0	19	229	M 459	6-25	362	2	0	24	336
M 528	6-29	315	0	2	41	272	M 534	6-29	370	7	0	41	322
M 462	6-29	207	0	2	19	186	M 457	6-29	278	7	2	26	243
M 531	7-3	256	0	0	32	224	M 519	7-3	91	4	1	4	82
M 459	7-3	347	0	1	22	324	M 504	7-3	197	8	0	17	172
M 534	7-7	366	1	10	25	330	M 528	7-7	360	9	1	53	297
M 457	7-7	308	6	4	22	276	M 462	7-7	271	6	2	44	219
M 535	7-11	235	0	0	11	224	M 532	7-11	270	26	4	45	195
M 464	7-11	202	0	1	24	177	M 461	7-11	416	12	2	92	310
M 531	7-15	339	8	0	32	299	M 528	7-15	297	21	1	26	249
M 461	7-15	293	1	2	28	262	M 464	7-15	121	7	0	11	103
Total		5435	18	49	408	4960	Total		5349	122	25	474	4728
Check treatment													
M 519	6-11	184	0	0	11	173	M 532	6-11	286	0	4	20	262
M 460	6-11	263	0	1	13	249	M 505	6-11	233	0	0	4	229
M 534	6-15	314	0	2	13	299	M 535	6-15	286	0	1	10	275
M 455	6-15	295	0	0	3	292	M 458	6-15	228	0	4	4	220
M 532	6-19	246	0	3	21	222	M 519	6-19	191	0	1	14	176
M 505	6-19	174	0	1	3	170	M 460	6-19	274	0	0	11	263
M 535	6-23	300	0	0	7	293	M 534	6-23	325	0	0	23	302
M 458	6-23	254	0	0	9	245	M 455	6-23	316	0	0	11	305
M 519	6-27	223	0	0	22	201	M 531	6-27	308	0	0	10	298
M 504	6-27	289	0	1	4	284	M 459	6-27	361	0	0	41	320
M 528	7-1	351	0	0	17	334	M 534	7-1	380	0	0	26	354
M 462	7-1	260	0	0	18	242	M 457	7-1	298	0	0	7	921
M 531	7-5	243	0	0	7	236	M 519	7-5	110	0	0	10	100
M 459	7-5	292	0	0	19	273	M 504	7-5	277	0	0	7	270
M 534	7-9	358	0	1	22	335	M 528	7-9	373	0	0	12	361
M 457	7-9	287	0	1	6	280	M 462	7-9	264	0	1	35	228
M 535	7-13	189	0	0	13	176	M 532	7-13	257	0	0	39	218
M 464	7-13	150	0	0	3	147	M 461	7-13	407	0	0	7	400
M 531	7-17	364	0	0	23	341	M 528	7-17	416	0	0	11	405
M 461	7-17	351	1	0	8	342	M 464	7-17	221	0	0	9	212
Total		5387	1	10	242	5134	Total		5811	0	11	311	5489

[a] Mating numbers between M 455 and M 505 include the queens from the susceptible line, and those between M 519 and M 535 include the queens from the resistant line.

Table 2.—Total numbers, percentages, and chi-square comparisons of dead, missing, and healthy individuals when nursed by adults from different lines within different treatments.

Nurse Bees[a]	Base Count	Observed Dead: AFB	Observed Dead: Unknown	Missing	Healthy Pupae at Final Count
Spore treatment					
R.N.B. No.	5435	18	49	408	4960
%		0.33	0.90	7.51	91.26
S.N.B. No.	5349	122	25	474	4728
%		2.28	0.47	8.86	88.39
Chi-square[b]		79.972	7.461	6.587	24.313
P=		<0.001	<0.01	0.01	<0.001
Check treatment					
R.N.B. No.	5387	1	10	242	5134
%		0.02	0.19	4.49	95.30
S.N.B. No.	5811	0	11	311	5489
%		0.00	0.19	5.35	94.46
Chi-square			0.004	4.397	4.004
P=			>0.99	.05–.02	.05–.02

[a] R.N.B. = "resistant" nurse bees, S.N.B. = "susceptible" nurse bees.
[b] Chi-square comparisons are between nurse bees within treatments. d.f. = 1.

diseased with American foulbrood, in due time it would be expected that the symptoms of the disease would appear in certain of these colonies. In the case of the resistant colonies a small number would be expected to develop the disease, whereas most of the susceptible colonies would be expected to develop it. Such considerations indicate that the results obtained in this experiment have biological and practical significance.

In the class of unknown dead in the spore treatment 49 (0.90%) were recorded in the samples nursed by "resistant" adults and 25 (0.47%) in the samples nursed by "susceptible" adults giving a chi-square of 7.461 with P= less than 0.01. When the total number (74) dying of unknown causes in the spore treatment is compared with the total number (21) recorded as unknown dead in the check treatment, there is suggested a possibility that some of these dead in the spore treatment might have died of American foulbrood. Had laboratory techniques of diagnosis been used in addition to field diagnostic methods, undoubtedly more of these unknown dead could have been classified as infected with American foulbrood. More individuals were recorded as missing among the combs of brood nursed by "susceptible" adults, than were recorded in the comparable brood nursed by "resistant" adults. This difference is not significant at the 1% level.

When the healthy pupae at final count are considered it is seen that 91.26% of the individuals remained healthy in the spore-fed colony nursed by bees from the resistant line compared to 88.39% which remained healthy in the spore-fed colony nursed by bees from the susceptible line. A chi-square comparison gives a value of 24.313 with P= less than 0.001.

For the sake of comparison, data for the check treatment also are included in table 2. It is seen that, between nurse-bee groups, there is no difference in unknown dead, missing, and healthy pupae when the 0.01 point is chosen as the level for significance. The data presented in this table indicate that adult bees from the resistant line were better able, by some inherent means, to keep the larvae which they nursed from succumbing to American foulbrood, than were adults from the susceptible line.

The phenomenon, reported here, of differential mortality in similar larvae subjected to identical inoculations but nursed by different adult bees may find its explanation in either of at least two hypotheses. The two considered most promising are: removal of pathogen from the sirup by the adult bees; and secretion of brood food having bacteriostatic and/or bactericidal properties.

Sturtevant & Revell (1953) reported on the numerical reduction of *Bacillus larvae* spores in liquid food of the bee by action of the stomach-mouth, or honey-stopper mechanism. This mechanism was operating in all lines of bees which they tested, but its operation was more efficient in some than in others. In the work reported herein, a more efficient action of the honey-stopper mechanism in the resistant line than in the susceptible line may have been the origin of the differences observed. On the other hand the resistant line may have been producing some substance in the brood food with bactericidal or bacteriostatic properties. McCleskey & Melampy (1939) stated that royal jelly does possess bacteriostatic and/or bactericidal properties against some organisms. Unfortunately *Bacillus larvae* was not among those used in their investigation. Holst (1946), however, reports that "royal jelly has been shown to exhibit a bactericidal effect on *Bacillus larvae*" on artificial culture media. But "the antibiotic action of royal jelly upon bacteria on artificial culture media may be completely different from that within the digestive tract of the honey bee larvae."

Most of the experimental larvae remained healthy, even in the colony nursed by adults of the susceptible line despite the fact that they were cared for, from the very moment of hatching, by nurses which had continuous access to the infective agent. It seems unlikely that such a large percentage of the larvae could be considered as "escapes" as Woodrow (1941a) has suggested, since the larvae received all their food from nurses which probably consumed the infectious sirup. It would appear that while so partaking of the infectious food the mouth-parts of the nurses would become contaminated. In the nursing process, such mouthparts would be expected to be a source of infection to the larvae, since it appears physically impossible for the nurse bees to be able to remove all spores from their mouthparts without some chemical or physiological aid. Such aid might come from a factor present in royal jelly, in saliva, or from some other material produced by the nurse bees. In addition to the antibiotic factors in royal jelly reported by McCleskey and Melampy, and by Holst, Philipp (1928) has described a varnish-like substance produced by the bee which he calls balm. This is used by the bees to coat each cell before it becomes the cradle of a new individual. He stated that this substance probably serves an important purpose in disinfection of all surfaces of the hive, especially the interior of the cells. A coating of such a material might explain why Woodrow (1941b) found healthy brood being reared in cells appearing to be clean, but yet containing *Bacillus larvae* spores. Such a material might also be used by the bee in cleaning her mouthparts, or accidentally get on the critical surfaces of the mouthparts while the bee polishes the cells thus disinfecting the mouthparts. Much remains to be learned in this field of study.

It has previously been stated that some of the sirup fed

Table 3.—Disease counts in experimental colonies subsequent to being fed spore-contaminated sirup stored by adults of resistant and susceptible lines.[a]

Date (Sept.)	Counts from Sirup Stored by "Resistant" Adults				Counts from Sirup Stored by "Susceptible" Adults			
			Daughter of			Daughter of		
	M 457	M 463	M 464	Total	M 455	M 458	M 505	Total
2	0/1[b]	0/0	0/1	0/2	0/16	0/38	0/2	0/56
4	1/0	1/0	0/1	2/1	2/0	0/31	3/2	5/33
6	3/0	0/0	0/0	3/0	0/0[c]	0/21	2/3	2/24
8	3/2	1/0	0/2	4/4		9/27	5/4	14/31
10	4/2	0/0	0/2	4/4		10/21	6/4	16/25
Total				13/11				37/169

[a] Experimental colonies were established on August 19, and were fed experimental sirup on August 21.

[b] The numerator represents the number of cells containing AFB, while the denominator represents the number of dead of unknown causes. Some were possibly AFB.

[c] Colony was weak with very little brood and was removed from the experiment.

was stored. It would seem to be of considerable interest to know whether the stored sirup would cause American foulbrood in other colonies, and whether the sirup stored by bees of the resistant line in the original experiment would differ from that stored by bees from the susceptible line in the production of foulbrood. With this idea in mind the stored sirup was extracted and fed to six small colonies of susceptible stock during the summer dearth of 1954. The mated queens heading these colonies were from the same susceptible line as that used in the 1953 experiment. Three were fed sirup stored by adults of the resistant line, and three were fed sirup stored by the susceptible line.

The results of this study are given in table 3. The number dead of American foulbrood is not great in any colony but when each group of three colonies is considered collectively, a difference is noted in the amount of American foulbrood observed. This suggests that the amount of infectious material present in the two lots of stored sirup was different. The adult bees of the resistant line may have been able, by action of the honey-stopper mechanism previously mentioned, to remove more of the spores from the sirup before storing it, than were the adults of the susceptible line; or the adult bees of the resistant line may have been equipped to destroy more of the spores by some unknown bactericidal means, than were the adult bees of the susceptible line.

It is concluded that there was a difference between adults of the two lines of bees under study, the difference being in the ability of the adults to protect the larvae of the colony from succumbing to American foulbrood. This difference was small, it is admitted, but it must be remembered that the stocks used had not been highly selected for resistance or for susceptibility. Whether this type of resistance is characteristic of all resistant lines is a matter of importance. The results obtained in this study suggest the need for further investigation in this field.

SUMMARY.—In the experiment presented, two colonies contained resistant-line adult bees and two colonies contained susceptible-line adult bees. Similar experimental brood was supplied to all four colonies, in an unbiased manner, from other resistant- and susceptible-line colonies. One resistant-line colony and one susceptible-line colony were fed *Bacillus larvae* spores in sirup. The other two colonies received sirup only, as a check treatment.

Observations were made on about 5000 larvae, distributed through 20 combs, in each colony. Survivors and non-survivors were tabulated. Non-survivors were further classified as dead of American foulbrood, unknown dead, or missing.

Results and their analyses showed no differences between the two types of adult bees in the check treatment. By the chi-square test, highly significant differences were found between adult bees in the spore treatment. Larvae nursed by resistant-line adults showed a lower incidence of American foulbrood and greater survival than similar larvae nursed by susceptible-line adults. This differential protection of larvae by adults may be due (among other possibilities) to removal of spores from the sirup by the honey-stopper mechanism, or to an anti-foulbrood factor in the larval food secreted by the nurse bees.

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Demeton Residues on Fruits, Vegetables, and Forage Crops[1]

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There is widespread interest in the use of the systemic insecticide demeton, for the control of insects and mites on many crops used for human consumption and livestock feed. During 1953, 1954, and 1955 a study was made at the Yakima, Wash., laboratory to obtain information on residues and their persistence in many kinds of crops, as determined by cholinesterase inhibition *in vitro.*

SAMPLING METHODS.—The method of sampling varied with the crop. A minimum of 2 pounds was used in all except tree-leaf samples, and samples representative of the entire plot or field were selected.

Four trees were sampled individually in each of the experimental plots of apples and pears at Yakima, and 25 fruits were selected from all parts of each tree. At harvest 15 apples or pears were taken at random from the boxes immediately after picking. Apple, apricot, peach, and pear samples from plots other than those at Yakima consisted of 50 or more fuits picked at random from the trees. Samples of prunes consisted of about 10 pounds of fruit.

Cantaloups were sampled by picking six at random from each plot. The rind was removed, before analysis, to leave a thin layer of the green flesh with the edible portion.

Each clover sample consisted of 2 pounds of the entire plant cut at random throughout the field.

Samples of pea vines consisted of 50 vines picked at random in each plot. The clover and pea vine samples were packed in dry ice for shipment to Yakima and were kept frozen until analyzed.

Peas harvested from the same plots as the vines were kept frozen until analyzed. They were shelled while frozen, and the peas and pods were analyzed separately.

Broccoli from experimentally treated plots was processed in a commercial freezing plant and packed in standard 12-ounce packages. Two or more packages were selected for each sample.

Each sample of table beets consisted of about 2 pounds of the roots, pulled at random throughout a plot, then washed and analyzed without freezing.

Potato samples consisted of 5 to 10 pounds of tubers dug at random and handled in the same manner as the beets.

Each sample of apple, pear or cherry leaves consisted of 25 leaves picked from all parts of a single tree. The leaves were kept frozen until analyses could be made.

METHOD OF ANALYSIS.—The cholinesterase inhibition method developed by J. Hensel and coworkers of Chemagro Corporation (unpublished), which is a modification of the method of Giang & Hall (1951), was used for all crops except potatoes. Another method developed by them, slightly modified, was used for potato tubers.

All samples were cut into small pieces and macerated in a blender, with the minimum amount of water necessary to obtain thorough grinding. The resulting slurry was filtered through paper, and an aliquot was then carried through the analytical procedure. An analytical filter aid (Celite) was added to the apple leaves.

For potatoes only, the following modification of Hensel's method was used: About 0.3 gram of sodium chloride per milliliter of filtrate was added, and the solution brought to a pH of 2 with 1 N hydrochloric acid. The solution was then blended with 3 volumes of chloroform. The mixture was poured into a separatory funnel, the chloroform layer drawn off, and the aqueous part again extracted by blending with 3 volumes of chloroform. The procedure was repeated again, the combined chloroform extracts were dried with anhydrous sodium sulfate, and filtered. The filtrate was transferred to a beaker and the chloroform evaporated on a steam bath with a gentle current of air blowing across the top of the beaker. When the solvent was nearly evaporated, about 0.5 ml. of water was added and the remainder of the chloroform removed. The sample was then transferred to a 10-ml. volumetric flask with a small amount of water, and the determination was carried out in the usual manner.

Standard demeton was added to the appropriate extracts from untreated check samples, at three different concentrations, and the cholinesterase inhibition determined simultaneously with each set of treated samples. A standard curve could then be plotted for each set of samples analyzed, thus eliminating the error that might result from day-to-day variation in the reaction.

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[2] The following workers applied the sprays and furnished samples for analysis: Washington Agricultural Experiment Stations—E. W. Anthon and W. J. O'Neil (Wenatchee), E. P. Breakey (Puyallup), P. M. Eide (Mt. Vernon) and K. E. Frick (Prosser); USDA Entomology Research Branch—W. C. Cook (Walla Walla, Wash.), F. P. Dean (Yakima, Wash.), O. A. Hills (Phoenix, Ariz.), B. J. Landis (Union Gap, Wash.), and H. W. Prescott (Forest Grove, Oreg.).

Table 1.—Demeton residues in various crops in Washington, Arizona, and Oregon.

Year and Locality	Crop	Ounces of Demeton per Acre	Number of Applications	Residue	
				Days After Last Application	Parts per Million
1953					
Mt. Vernon, Wash.	Table beets	10.2	6	49	2.2
		6.8	4	7	20
1954					
Phoenix, Ariz.	Cantaloupe	4.0	1	8	0
				18	0
				26	0
Forest Grove, Oreg.	Clover	14.2	1	4	Plot 1, 149
					Plot 2, 150
				14	60
					45
				31	17.5
					3.1
Prosser, Wash.	Cherry (leaves)	3.4	1	0	69
				3	48
				7	44
				10	44
				14	40
				94	14
Puyallup, Wash.	Loganberries	6.8	3	34	2
Walla Walla, Wash.	Peas	6.0	1	0	Vines 71.1
				7	16.5
				14	Tips 1
					Lower part 8.3
				26	Peas 0
					Pods 0
Prosser, Wash.	Potatoes, Netted Gem	3.0	3	53	0.2
		6.0	3	53	0.4
		12.0	3	53	0.3
Wapato, Wash.	Potatoes, Bliss Triumph	3.0	2	22	0.05
				38	0.10
Puyallup, Wash.	Strawberries	3.4	1	22	0.9

RESULTS.—Data for the different crops are given in table 1. It will be noted, in general, that leafy crops contained much larger amounts of demeton than root crops and fruits. The test in which pea vines were sampled to permit analysis of the new growth and the older parts of the vines separately indicated that the concentration of demeton was highest in the older leaves.

Winesap apple treees were sprayed three times in 1954 and twice in 1955 with 1.3 ounces of demeton in 100 gallons, and pears were sprayed twice in 1955 with the same concentration at Yakima. At various intervals after spraying analyses were made to determine the disappearance rate in the leaves and whether there was any correlation between demeton concentration and the control of mites and aphids. Since the amount present in the fruits at harvesttime was of particular interest, analyses were made at that time. Complete data are shown in table 2.

Table 3 shows the demeton residues found in fruit sprayed at Wenatchee, Wash. The amounts found were roughly proportional to those applied.

DISCUSSION.—The application of demeton to fruits and vegetables results in low concentrations of the material in fruits, roots, and tubers. The material is concentrated in the leaves of the various plants and there is little movement to new growth after the first few days following application.

There is some evidence of downward movement of demeton in potatoes and table beets, since the pesticide was detected in the tubers and roots when the spray was applied only to the foliage.

The persistence of a high concentration of demeton in apple leaves for almost 2 months after application was unexpected, and in sharp contrast to amounts found in leaves from other plants. Metcalf *et al.* (1955) have shown that the principal toxic metabolites, in plants, are probably the thiolphosphate sulfoxide and the thiolphosphate sulfone. These thiol isomers accumulated five to ten times as rapidly as products resulting from treatment with the thiono isomer. Furthermore, the thiol isomers were five to ten times more effective as cholinesterase inhibitors than the technical demeton. It was also shown that the thiol isomers could persist for different lengths of time in different plants. It appears probable, therefore, that

Table 2.—Demeton residues in apples and pears, Yakima, Wash.

Sample	Year	Residue: Days After Spraying[a]	Residue: Parts per Million
Leaves:			
Apple	1954	First	
		0	88
		1	102
		3	10
		4	70
		7	143
		Third	
		56*	54
	1955	First	
		0	72
		7	246
		16	101
		Second	
		0	161
		6	230
		13	155
		21	203
		34	197
		48	108
		55	56
Pear	1955	First	
		4	118
		6	70
		7	35
		Second	
		0	35
		70	1.2
Fruit:			
Apples	1954	Second	
		0	0.35
		13	.76
		21	.37
		Third	
		5	.46
		56*	.28
	1955	Second	
		80*	.2
Pears	1955	80*	0

[a] All sprays contained 1.3 oz. of demeton in 100 gallons of water. * Indicates harvest.

conditions in apple leaves permit a high rate of accumulation and a slow rate of metabolism of the thiol isomers.

Table 3.—Demeton residues in fruit, Wenatchee, Wash.

Fruit	Ounces of Demeton per 100 Gallons	Number of Applications[a]	Residue: Days After Last Application[b]	Residue: Parts per Million
Apricots	3.4	1 (1954)	49	0.18
			77*	.17
Apples:				
Delicious	6.8	3	90	.90
Jonathan	1.7	3	73	.30
	3.4	3	73	.40
	5.8	3	73	.70
Winesap	40.8	1	15	5.3
Peaches:				
Hale	3.4	2	1	1.4
			6	1.7
			19	1.1
			29	.8
	5.1	2 (1954)	8	3.8
			25	3.0
			32	2.3
			49	1.6
	40.8	1	4	3.8
Halehaven	6.8	1	1	1.5
			6	1.8
			19	2.4
			29	1.1
Pears:				
Bartlett	1.7	3	67	0
	3.4	3	67	.3
	6.8	3	67	.3
d'Anjou	1.7	2	99*	.17
	6.8	2	99*	.4
Prunes, Italian	3.4	2	1	.8
			7	1.3
			19	.8
	5.1	1 (1954)	107	.4
	6.8	2	1	3.0
			7	2.4
			19	2.8
	40.8	1	3	5.4

[a] 1953 unless otherwise indicated.
[b] * indicates harvest.

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Pyrethrum Emulsions for Surveys of Tabanid Larvae[1]

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Several years ago workers in Massachusetts showed that surface applications of a pyrethrum-kerosene emulsion (Bailey 1948) or a solution of sodium lauryl sulfate, thiodiphenylamine, and pyrethrum in petroleum distillate (Khan 1951) forced tabanid larvae to migrate from the soil. Since larval surveys are an integral part of the biological study of tabanids, it was believed that similar treatments might supplant the tedious and time-consuming mechanical survey and collection methods. During the last 2 years pyrethrum emulsions, with and without piperonyl butoxide, and lindane emulsions have been applied to selected natural habitats of tabanid larvae to evaluate the technique under Florida conditions.

MATERIALS AND METHODS.—The pyrethrum formulations used in the tests were (1) a commercial emulsifiable concentrate containing pyrethrins and piperonyl butoxide (Pyrenone T-143), (2) a specially formulated 1:10 pyrethrins-piperonyl butoxide concentrate containing CTX-54 (a blend of nonionic and anionic surfactants) as the emulsifier and Aroclor 1248 (chlorinated polyphenyls) as described by Richards (1956), and (3) a similar specially formulated concentrate but without the synergist. A commercial emulsifiable concentrate containing lindane was also used.

Test areas were selected near Gainesville, Fla. along the margins of fresh-water lakes and streams and in bogs and marshes where previous sampling had shown tabanid larvae to be present. Plots 1 square yard in area were cleared of vegetation and spaced so that two or more plots could be treated and observed at the same time.

Two methods of application were used, surface applications and injections. In both methods the emulsions were applied at the rate of ½ gallon per square yard. The surface applications were made by sprinkling or spraying the materials evenly over the entire plot or on the roots and surrounding soil of uprooted aquatic and semiaquatic plants. A sprinkling can and a portable pneumatic sprayer appeared equally suitable for this purpose. For the injections the conventional spray nozzle of a portable pneumatic sprayer was replaced with a modified soil-fumigation nozzle which was inserted 3 or 4 inches below the soil surface. This attachment gave lateral penetration of 6 to 12 inches when the sprayer was operated at approximately 40 pounds' pressure, thus spot treatment every 12 to 18 inches gave adequate coverage.

After treatment migrating larvae were picked up, placed in clean water, and then transferred to ½-pint jelly dishes containing uncontaminated soil. Since it was known that larval populations vary considerably in different plots, it was necessary to establish a population average for each plot. Therefore, three soil samples, each 1 square foot and 2 to 3 inches deep, totaling one-third of the plot, were taken at random from each plot after 1 hour had been allowed for the larvae to appear at the surface. The number of larvae recovered from these samples was multiplied by 3 and then added to the number of larvae that were forced to the surface by the treatment to give the population average. The percentage surfacing for each plot was calculated from the population average.

SURFACE APPLICATIONS.—The commercial and specially formulated pyrethrum-piperonyl butoxide formulations were compared at three concentrations in three separate areas. As shown in table 1, the laboratory formulation was equal to or better than the commercial formulation in all tests. Although the laboratory formulation gave higher surfacing percentages in all tests, both gave more consistent results in area 3 where the larvae were more abundant. The better results obtained with the laboratory formulation can probably be attributed to the fine colloidal texture of the emulsion. It gave excellent soil penetration, with puddling limited to very wet places and those soils with a tight clay surface. The commercial formulation, used primarily as a livestock spray, made a coarse emulsion and uniform penetration was difficult to obtain except on coarse-textured soils.

The larvae obtained in these tests were primarily *Tabanus atratus* F. and *fumipennis* Wied., although *lineola* F. and a single specimen of a species near *nigripes* Wied. were also surfaced. *T. petiolatus* Hine and *Chrysops flavida* Wied. were also present in the screen-dried samples, but these species were not flushed from the soil. Larval mortalities in these tests were negligible.

The laboratory formulations were also tested to determine whether or not the addition of piperonyl butoxide contributed to the surfacing efficiency of pyrethrum. The results of these tests, presented in table 2, show that the synergist reduced the surfacing time and afforded somewhat better surfacing percentages at all concentrations. The species obtained were *T. atratus*, *fumipennis*, *lineola*, *nigripes*, and *petiolatus*, and *C. flavida*, and *vittata floridana* Johns. The *Chrysops* larvae that surfaced died within 24 hours after collection, but in the other species that surfaced there was no mortality that could be attributed to the chemical treatments.

The specially formulated pyrethrins-piperonyl butoxide emulsion was applied to three species of aquatic plants at a concentration of 0.001% of pyrethrins. Ten plants each of *Peltandra*, *Carex*, and *Panicum* were used. The plants were uprooted, and after a brief drainage period the emulsion was sprinkled on the roots and surrounding soil. Of the larvae found in *Peltandra*, 62% were driven out, as compared with 40% out of *Carex* and 45% out of *Panicum*. The species flushed from the plants were *T. fumipennis*, *stygius* Say, and *imitans excessus* Stone. Post-treatment sieving showed that these species were the only tabanids in these habitats.

Emulsions containing 0.5% of lindane were applied to seven test plots. The treatments were made in the usual manner, but the larval response was very slow. An hour or more was required for the first larva to appear at the surface; then surfacing continued for 24 hours or more. All the larvae that reached the surface died within 72 hours after collection, and many were immobilized in the

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[2] In cooperation with the University of Florida Agricultural Experiment Station, Gainesville.

Table 1.—Effectiveness of surface applications with two 1:10 pyrethrum-piperonyl butoxide emulsions in forcing tabanid larvae from the soil.

Area	Number of Larvae per Square Yard	Pyrethrins (Per Cent)	Per Cent of Larvae Surfaced	
			Commercial Formulation	Laboratory Formulation
1	9	0.004	0	67
		.002	0	22
		.001	11	22
2	6	.004	17	33
		.002	0	0
		.001	0	17
3	14	.004	14	50
		.002	32	50
		.001	7	7

soil and failed to reach the surface. Of the larvae found in four soil samples examined 48 hours after treatment, 66% were dead. The living larvae were removed and placed in clean sand, but they died within 72 hours. The species surfaced by the lindane treatments were *T. atratus, fumipennis*, and a few specimens of *petiolatus;* these specimens were full grown or nearly so. No attempt was made to determine the species of dead larvae found in the soil samples. Owing to the very slow action of lindane and resulting high larval mortalities, further attempts to develop it as a survey tool were deemed impractical.

The successfulness of flushing larvae from the soil by surface applications of pyrethrum emulsions probably depends on many factors, but those affecting insecticide penetration into the soil appear to be the most important. Two such factors are the amount of water and the physical character of the habitat. Very wet places proved to be poor test sites, and tight-textured soils afforded poor penetration of the emulsions and gave disappointing results. The best surfacing percentages were obtained along the edges of small streams and shallow lakes where the larval habitats were primarily sand.

The different species also vary in sensitivity to the treatments. Although nine known species were driven from the soil by surface treatments of pyrethrum, only three species were surfaced in appreciable numbers. The larvae of *T. atratus, fumipennis*, and *lineola* are very active, and thus readily come to the surface when chemically irritated. The less active larvae of *T. nigripes* and *petiolatus* and *C. vittata floridana* and *flavida* were surfaced only in small numbers even in the habitats where these species predominate. *T. stygius* and *imitans excessus* were flushed only from the roots and surrounding soil of uprooted aquatic plants.

Tests conducted during the winter months indicated that soil temperature may also be an important factor. Negative or very poor results were obtained when the emulsions were applied at soil temperatures lower than 55° F.

The mortality appeared to be directly correlated with the size of the larvae concerned in the test. *Tabanus* larvae, one-third grown or more, rarely showed adverse effects when they were removed from the treated soil and immediately transferred to clean water and then to clean soil. Full-grown *Chrysops* larvae usually survived the treatments, but mortalities were often high where smaller specimens were concerned. Although there were some deaths of smaller specimens of both *Tabanus* and *Chrysops*, most of the surfaced larvae were successfully maintained for biological studies, and many of them were reared to adults.

INJECTION APPLICATIONS.—The failure of surface applications to penetrate the soil under certain conditions prompted further studies in which the laboratory formulations were injected into the soil. Two series of tests were conducted, in the fall of 1954 and the spring of 1955. The results, presented in table 3, show that the response to injections was not so high as to surface applications. However, all these tests were made in situations where surface applications had shown near or complete failure. Larval mortalities ranged from 39 to 100% in the first series of tests and from 0 to 60% in the second series. The lower mortalities in the second series were probably due to the larger size of the larvae, which were full grown or nearly so. The addition of piperonyl butoxide increased the surfacing percentages but caused higher mortalities.

In all the treated plots many of the larvae were immobilized in the soil and never reached the surface. That the injected emulsions penetrated deeper into the habitats than those applied to the surface was evidenced by the fact that *T. nigripes* and *petiolatus* were surfaced in nearly the same numbers of *atratus* and *fumipennis*. In addition,

Table 2.—Surfacing of tabanid larvae from surface applications of an emulsion containing pyrethrum alone and combined with piperonyl butoxide.

Pyrethrins (Per Cent)	Number of Plots	Surfacing Time (Minutes)	Mean Per Cent Surfaced
Alone			
0.002	3	15–60	68
.001	5	20–90	76
.0005	2	15–120	40
With synergist (1:10)			
.002	4	10–45	84
.001	4	10–45	82
.0005	5	15–60	52

Table 3.—Surfacing of tabanid larvae from injection applications of an emulsion containing pyrethrum alone and combined with piperonyl butoxide.

Pyrethrins (Per Cent)	Alone		With Synergist (1:10)	
	Number of Plots	Per Cent Surfaced	Number of Plots	Per Cent Surfaced
	Fall, 1954			
0.002	—	—	4	61
.001	5	22	6	26
.0005	3	0	4	16
	Spring, 1955			
.002	4	28	6	48
.001	4	11	6	33

larvae of *T. gladiator* Stone and *Diachlorus ferrugatus* (F.), species not flushed from the soil by surface applications, responded to the injection treatments.

The primary objections to this method were high larval mortalities and lower surfacing percentages. However, the injections showed definite value for population and distribution studies in very wet areas and other situations where surface applications were unsuccessful.

SUMMARY.—Tests were conducted in the vicinity of Gainesville, Fla., to evaluate soil treatment with insecticides as aids in tabanid larval surveys and collections. Plots were treated with emulsions from three pyrethrum formulations—a commercial pyrethrins-piperonyl butoxide concentrate and two specially formulated concentrates, with and without piperonyl butoxide—and also with a commercial lindane concentrate. All the emulsions were applied as surface treatments, and the specially formulated emulsions were also injected into the soil.

Surface applications of pyrethrum emulsions forced tabanid larvae to the surface when soil conditions were suitable for emulsion penetration. Nine known species were surfaced. The laboratory formulation containing piperonyl butoxide was more effective than the commercial preparation. The lindane treatments were not so effective as pyrethrum for forcing larvae to surface, and all the affected larvae died.

Injection treatments showed advantages in very wet situations and other areas where there was poor penetration of the surface applications. The larvae of two additional species were surfaced by this method.

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Laboratory Studies on the Toxic Effects of *Therioaphis maculata* (Buckton)[1]

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The spotted alfalfa aphid, *Therioaphis maculata* (Buckton), since its discovery in the United States in 1954, has been found to be severely injurious to newly planted as well as mature alfalfa stands. Dickson *et al.* (1955) reported that damage to alfalfa resulted both from the removal of plant juices and apparently from the injection of a toxin. These workers also reported that alfalfa growth was retarded and that seedling plants, being especially susceptible, were sometimes killed by single aphids.

More recently, Diehl & Chatters (1956), studying the mechanics of feeding, showed that the insects inserted their stylets intercellularly and fed within both mesophyll and phloem tissues. When feeding, saliva, or other toxic substances, was injected. Damage was most evident in the mesophyll where feeding appeared to cause lysis of the cytoplasm. Such internal damage could be externally correlated with macroscopic, chlorotic areas on leaves. The authors concluded that the "inserted contaminant" was highly toxic and had a plasmolytic effect. Because of these reported toxic effects of the insects, a study of this problem was begun by the authors early in 1956 with especial emphasis on stunting and killing of seedling plants.

MATERIALS AND METHODS.—Test plants were alfalfa seedlings (*Medicago sativa* L. var. African) selected for uniformity after the emergence of the first true leaf. Foundation seed (1954), obtained from E. H. Stanford, Department of Agronomy, University of California, Davis, was sown in sterile sand, incubated for approximately 3 days at 30° C., and transplanted into 3-inch clay plots containing sterile soil. Sterile soil helped reduce losses due to damping-off, and resulted in better survival of plants used in the tests. As needed the plants were watered with ½-strength Hoagland's solution.

Test insects were spotted alfalfa aphid apterae, obtained from the insectary stock colonies. These cultures were initiated originally from 1955 field collected insects. Test individuals were selected at random from stock. Insect transfers were made with a micro-pipette. Placement on test plants was somewhat at random, position not being considered an important variable. During the period of exposure to insects, watering was done by means of clay saucers which also acted as a trap barrier for the occasionally wandering insect.

The exposure period to aphids in all experiments was 7 days, at the end of which time the aphids were killed with a nicotine sulfate spray. Daily records were made during the experimental period.

RESULTS.—*Preliminary.*—In January, 1956, a few apterous female spotted alfalfa aphids were obtained from H. M. Graham, Department of Entomology and Parasitology, University of California, Berkeley. This source colony was being maintained on a large field collected alfalfa plant. After acquisition, the insects were placed on a young alfalfa seedling. Within a week, yellow vein-banding symptoms (fig. 1) appeared on the two youngest leaves, and there were indications of stunting. It has since been confirmed through a large series of tests that seedling alfalfa plants, grown in the greenhouse and infested with spotted alfalfa aphids will rapidly develop a distinct yellow vein-banding of the leaves at or near the growing tip. In addition, as the infestation ages, a localized yellow

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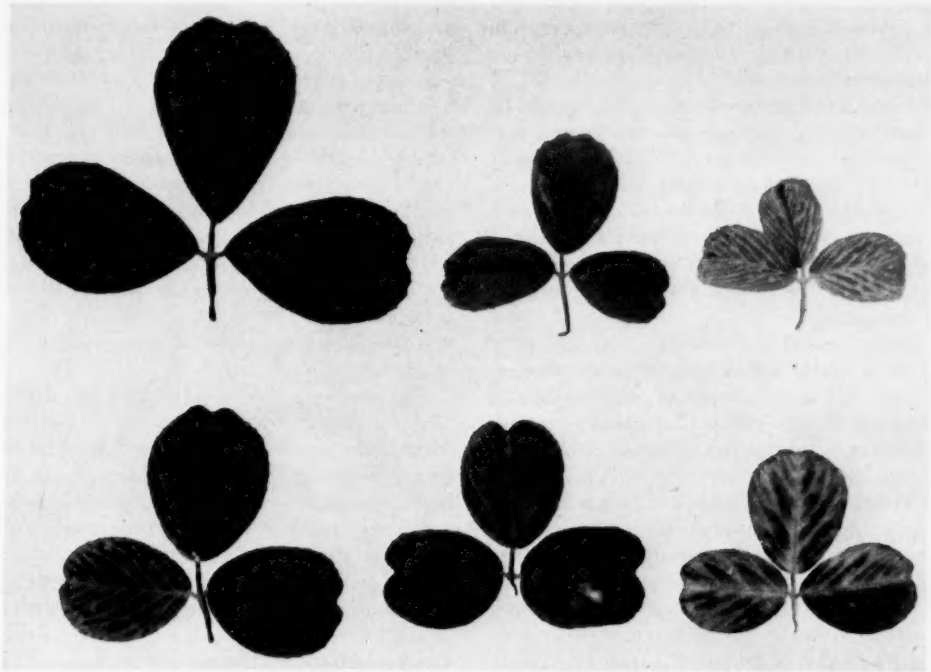

FIG. 1.—Yellow vein-banding symptoms on young alfalfa seedlings induced by the feeding of spotted alfalfa aphids.

spotting develops at the feeding site, presumably similar to that described by Diehl & Chatters (1956). Vein-banding subsequently has been observed on field alfalfa, especially where there is rapidly growing tissue.

Although the primary hypothesis upon which the work was planned was that the symptoms associated with the feeding of the spotted alfalfa aphid were due to a toxemia, the symptoms observed, especially the initial vein-banding, were similar to those which accompany some plant virus infections. Since the original aphids were colonized on a field-collected alfalfa plant, and since alfalfa, after being in the field for more than 1 or 2 seasons is known to serve as a host for numerous strains of the alfalfa mosaic virus, as well as other viruses, there remained the possibility that the symptoms observed in the first seedling plant used were the result of a virus transmitted by the aphids from the field-collected source plant. This possibility was investigated in a series of preliminary experiments.

Three phenomena were selected as being particularly useful in distinguishing between an insect-induced toxemia and a virus disease: 1) the inherent ability of the insects to induce the symptoms, 2) the recovery from symptoms after removal of the insects and 3) the brevity of the incubation period of the disease.

Inherent ability of the species to induce the disease.—To date this probably is the most definitive phenomenon used to separate a virus disease carried by aphids and a toxemia. Four apterae, selected from the colony established on the original *seedling* plant, were transferred daily, for 6 days, to new seedlings. At the same time, nymphs produced during each 24-hour period, were transferred to separate seedlings. During the serial transfers, the number of aptera decreased to two. The number of nymphs produced and transferred daily varied from 8 to 19.

In each of the six plants upon which nymphs alone had fed, yellow vein-banding developed. The results favored the toxemia hypothesis, since there is no substantiated case, to the authors' knowledge, where either: 1) aphid nymphs will acquire virus from a plant within 24 hrs. after inoculation, or 2) that an aphid-borne virus is passed transovarially.

Recovery from symptoms after removal of insects.—Although plants infected with a virus will, after the acute phase of the disease, frequently lapse into a chronic phase of lessened symptom intensity, complete recovery from symptoms is rare. Numerous observations during the present work support the conclusion that when spotted alfalfa aphids are removed from plants, the intensity of the vein-banding symptoms lessens, and subsequent new growth shows less and less evidence of disease. Eventually such plants recover.

If infested alfalfa plants are cut back, fumigated, and fertilized, the re-growth is entirely free of vein-banding symptoms. Normally, with virus disease, the opposite reaction occurs, for nutrient supplementation, coupled with servere pruning, typically intensifies symptoms. Exceptions exist, *e.g.*, beet yellows, but the authors are unaware of any exceptions associated with the type of symptoms involved in the case of alfalfa and the spotted alfalfa aphid. Again the evidence supports a toxemia hypothesis.

Brevity of the incubation period.—Systemic virus disease incubation periods, although variable, and somewhat dependent upon the rate of plant growth, usually are not less than 5 to 7 days. Curly-top virus disease is a notable exception, with an infrequent minimum incuba-

tion period of 2 days (Severin 1924). The generalization applies specifically to systemic infections, as local lesion reactions can occur within 48 hours.

However, the brevity of an incubation period might be used to help distinguish a systemic toxemia from a systemic virus infection. Severin *et al.* (1945) specifically contrasted the short incubation period of a toxemia of asters induced by the feeding of *Xerophloea vanduzeii* Law. with a disease caused by the aster yellows virus. Such a comparison was avoided in the case of sugar beets, presumably because of the reported 48-hr. minimum of the curly-top disease.

If the incubation period is defined as that period of time elapsing from initial aphid infestation of seedling alfalfa plants until the development of vein-banding at the apical portions of the plant, the results to date indicate that the incubation period is usually 48 hours in length, with a minimum of 24 hours (fig. 2). The rapidity of symptom development after initial infestation indicates the presence of a rapidly translocated substance, somewhat potent in action (or in high concentration), and one which is independent of any normal virus-like multiplication process. The assumed tissue of rapid translocation of viruses is phloem, with the xylem being used in some cases, *e.g.*, Pierce's disease of grapes. By analogy, it would appear that the toxin was being translocated by phloem tissues from the site of injection to regions of rapid growth.

The aphids did not need to feed for a full 24 hours to induce symptoms. However, in such cases, symptoms developed after insect removal. The vein-banding increased in intensity and/or extent as either the length of exposure, or the number of insects, was increased. This dependency of severity upon the amount of feeding again was suggestive of a toxemia rather than a virus disease (Carter 1935).

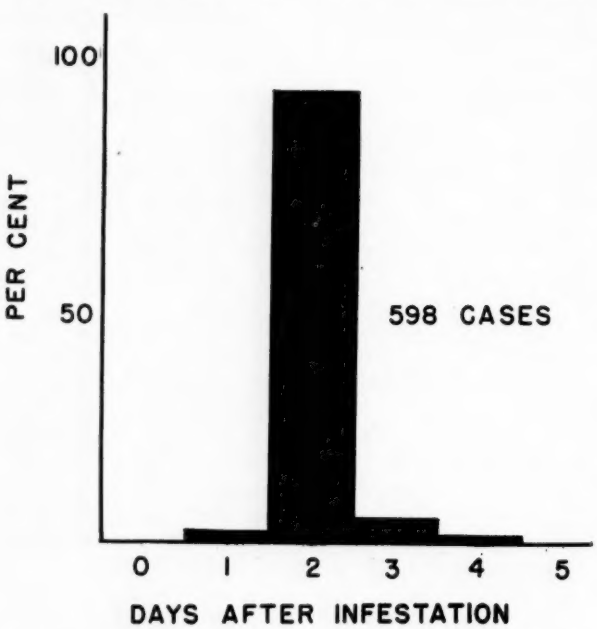

FIG. 2.—The incubation period required for the development of vein-banding symptoms on seedling alfalfa following infestation with spotted alfalfa aphids.

Index of plant stunting.—Two methods were compared to determine the amount of stunting caused by the spotted alfalfa aphid. It was believed that either total petiole length or the fresh green weight of test plants would give a suitable index of stunting. A sample of 20 plants was selected at random from a group of 40 which had been infested for 7 days with one aptera and progeny. Each plant was cut off at soil level, weighed to the nearest 0.1 mg., and meaured to the nearest 0.1 mm. for total petiole length. The mean ($\pm$standard deviation) weight was 65.2 ± 16.5 mg., while the mean length was 7.8 ± 2.2 mm.

The coefficient of variability for weight was 25%, and that for length was 28%. Both methods were deemed comparable as an index of plant stunting, with approximately the same degree of precision. Since measuring was more time consuming, weighing was selected as the standard procedure. The weighing time was initially shortened by using groups of five plants. Later, after acquiring a torsion balance, individual plants were weighed.

Effects of caging on plant growth.—In preliminary trials, seedlings were caged with 9-×32-mm. shell vials. Paper liners were found to be needed to enable dislodged aphids to crawl back onto the plants. To test the effects of caging on plant growth, the following caging conditions were compared on groups of 20 plants, with and without one aptera and progeny feeding for 1 week: glass vials with—1) a narrow strip of paper, 2) a half circumference paper liner, 3) a full circumference paper liner, and 4) a control with no cage. The results are given in table 1 and figure 3. The regression lines were calculated assuming the plotted data followed a second degree equation, $Y = a + bX - cX^2$, assigning the treatments the nonquantitative numbers 1, 2, 3, and 4, in order of increasing amount of paper and presumably decreasing amount of light.

The aphids caused a measurable amount of stunting in the absence of caging (L.S.D.0.05 = 5.17 mg.), but caging of plants without aphids resulted in some decrease in plant weight. Statistical support could be found for concluding that a measurable reduction in weight occurred through the use of vial cages with either a half or a full circumference paper liner.

The measured effects of aphid attack were comparatively less under the most unfavorable growing conditions. Presumably the effects of caging and aphid attack were not strictly additive. Maximum effect of aphid attack was when the cage influence was at its lowest value. In this treatment (vial cage+strip paper liner), probably there was some light reduction and increased humidity.

Table 1.—Comparison of the effects of cage types, with and without aphids for 7 days, on the growth of seedling alfalfa plants.

	Mean Fresh Green Weight (mg.)			
Presence of Aphids	No Cage	Cage+Strip Paper Liner	Cage+Half Circumference Paper Liner	Cage+Full Circumference Paper Liner
Without aphids	132.1	127.7	115.7	93.7
With aphids	83.9	65.2	53.3	52.4

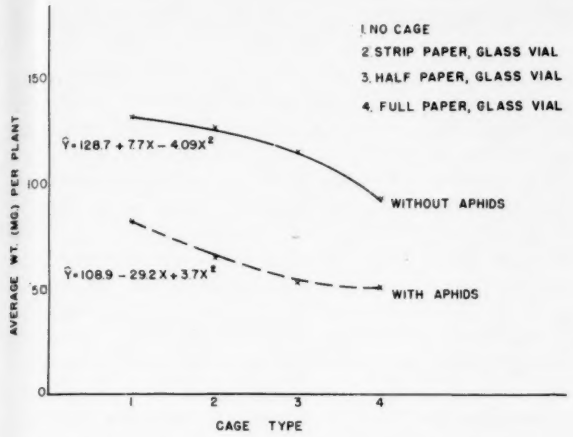

FIG. 3.—Effects of various cage types, with and without aphids for 7 days, on the growth of seedling alfalfa plants as measured in milligrams of fresh green weight.

Evidently plants were somewhat weakened by these conditions, even in the absence of aphids, but such weight reduction as did occur was not satistically supportable at the 5% (L.S.D.0.05 = 5.5 mg.).

Additional observation showed that when cages were removed at the end of the exposure period, the plants had a shock reaction, presumably due to rapid desiccation. The wilting persisted for approximately 48 hrs., and in some cases, where there was additional drying stress, individual plants died. Since cages reduced plant vigor, and since observations made during this time indicated that the majority of aphids remained on the plants during the exposure period without caging, all further experiments were done without cages.

Effects of aphid attack on alfalfa seedling growth.—

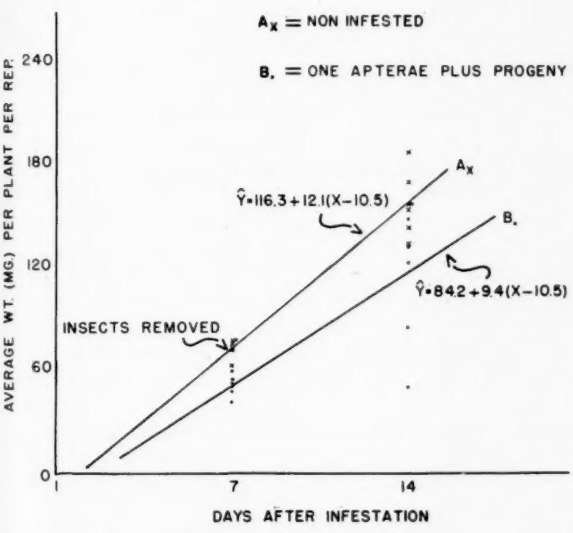

FIG. 4.—Reduction in fresh green weight of alfalfa seedlings over a 2-week period, the first week of which the plants were exposed to infestation by one apterous spotted alfalfa aphid and progeny.

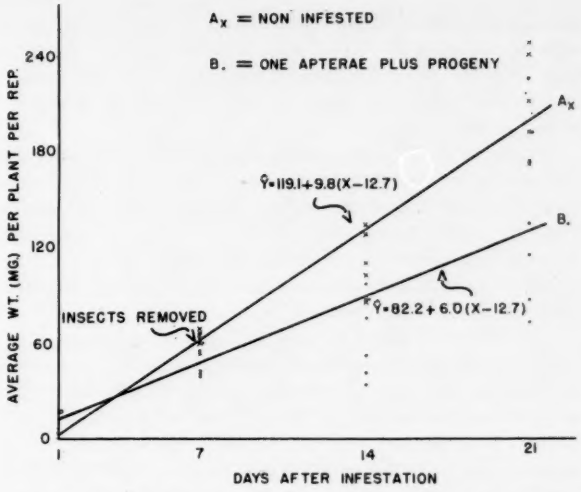

FIG. 5.—Reduction in fresh green weight of alfalfa seedlings over a 3-week period, the first week of which the plants were exposed to infestation by one apterous spotted alfalfa aphid and progeny.

Experiment I, done to determine the effects of spotted alfalfa aphid feeding on African variety alfalfa seedlings, was a randomized block design of 2 treatments, 40 plants per treatment, 2 growth period measurements, and 6 replications. Treatments were: 1) no aphids and 2) 1 aptera and progeny feeding for 7 days. The 2 growth periods sampled were: 1) 7 days after initial infestation, and 2) 14 days after initial infestation, or 7 days after removal of the aphids.

This experiment was not entirely satisfactory. An estimate of initial plant weight was not taken, and the 1-week recovery interval may not have been long enough to indicate the recovery trend. A second series of 6 replications was carried out using the same 2 treatments, with 3 growth period samples (1, 2, and 3 weeks after initial infestation). The initial weight (day of infestation) was estimated by weighing a sample of 40 plants selected from the population from which the experiment was set up.

The results of experiments I and II are graphically represented in figures 4 and 5, assuming linearity. Table 2 gives the results of analysis of variance. Significant evi-

Table 2.—Fresh green weight reduction due to the feeding of one apterous spotted alfalfa aphid and progeny for 7 days on alfalfa seedlings.

Experiment No.	Mean[a] Fresh Green Weight (Mg.) per Plant							
	Control on Date Shown				One Aptera+Progeny on Date Shown			
	1[b]	7	14	21	1	7	14	21
I	—	73.9	158.6	—	—	51.6	116.8	—
II	17.8	61.7	123.2	206.3	—	50.1	79.9	138.2

L.S.D. 0.05: Experiment I, 24.7; Experiment II, 27.2[c]

[a] Based on 40 plants per treatment, 6 replications in Experiment I; 60 plants per treatment, 6 replications in Experiment II, with the exception of day 1, which was estimated from 40 plants.

[b] Day of infestation, other periods are measured from this time; aphids removed after 7 days.

[c] For analysis of variance, only the 7, 14 and 21 day samples used.

dence was obtained for the following conclusions: One aptera and progeny caused a measurable amount of stunting. The evidence for total differences betweeen treatments was significant, but not when considered in any 1-week period.

Experiment II produced significant evidence for differences between treatments in all but the 7-day sample, where the effects of the toxemia on growth could not be statistically differentiated from noninfested plants. The differences became separable 14 days after infestation, and were more evident 21 days after infestation, or 14 days after removal of the aphids.

Association of plant damage with aphid numbers.—One source of variation was due to unequal numbers of aphids feeding on the test plants during the 1-week exposure period. In experiment I, of the 236 (out of a possible 240) plants weighed, approximately 20% (48 plants) had 1 aptera plus 1 nymph or less feeding for the 7-day infestation period. At the other extreme, about 8% (19 plants) had populations which peaked in the range of 25 to 35 aphids sometime during the 7-day infestation period. In Experiment II, in which 357 (out of 360 possible) plants were weighed, only 4% were plants which had 1 aptera plus 1 nymph or less feeding for the 7-day infestation period, while 11% had peak populations of 25 to 35 aphids. These differences in numbers of aphids feeding were partially responsible for high within-treatment variation.

It has been suggested that the severity of the toxemia is dependent on, or proportional to, aphid numbers. Since individual plants were not uniformly infested, it was not possible to compare tests using constant aphid numbers as the treatment variable. However, in three replications of experiment II, daily records were made on the number of aphids on each plant, and in addition each plant was weighed. Thus it was possible to plot individual plant weights (in mg.) versus aphid-days of attack, in order to partially test the hypothesis that plant damage was proportional to number of insects feeding. An aphid-day was defined as one aphid (aptera or nymph) feeding for 1 day.

Samples were taken 1, 2, and 3 weeks after initial infestation. A few plants were not considered in the analysis for such reasons as aptera death, failure to reproduce, or substitution of plants. These situations were judged as nontypical for comparative purposes.

The results comparing the 3 weeks are given in figure 6, with linearity being assumed. The highest degree of plant weight association with aphid-days of attack was found in the 3-week sample (2 weeks after release from infestation), with an r value of -0.485. Both slope and variability increased during the 3-week period. In the 1-week

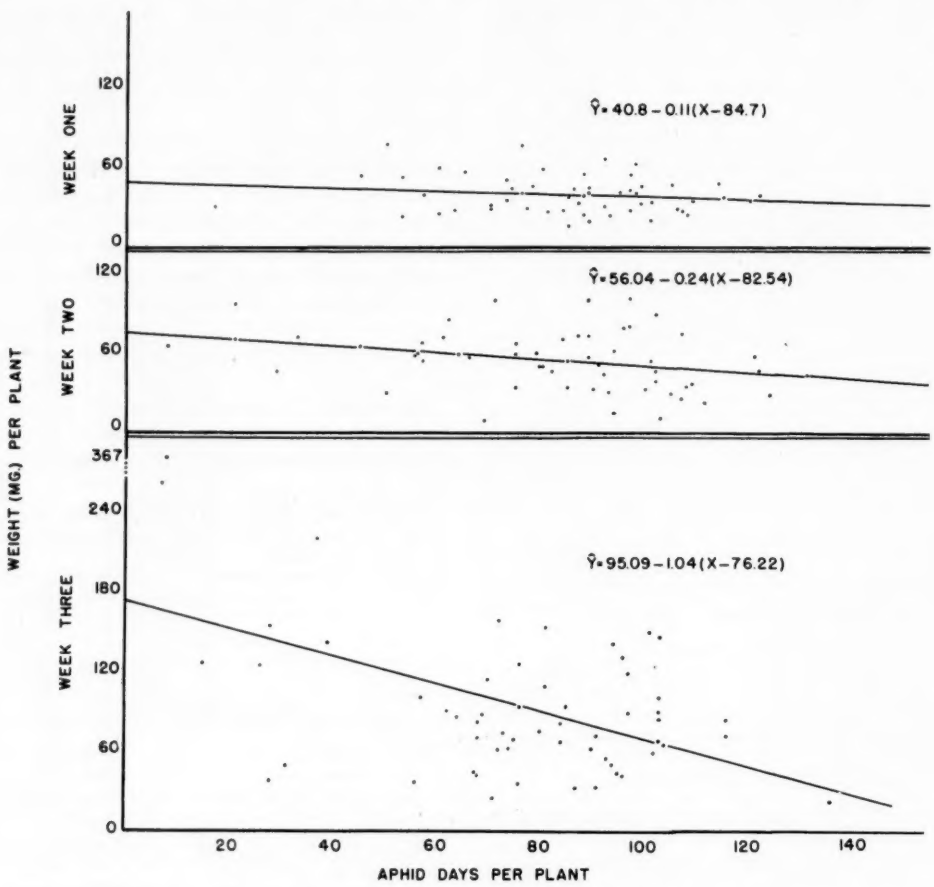

FIG. 6.—Influence of aphid-days of feeding on the fresh green weight of seedling alfalfa plants. Insects were removed after 7 days of feeding, and plant samples were taken 1, 2, and 3 weeks after initial infestation.

sample it was difficult to associate plant damage with the number of aphid-days of attack.

Statistical analysis, using t to test for deviation of the estimated slopes, $\hat{b}$, from assumed null hypothesis slope $\beta=0$, indicated that $\hat{b}=-0.107\pm0.09$ (standard error) for the regression line of week 1, was not sufficiently different from $\beta=0$, to reject the null hypothesis ($t=-1.18$, d.f.$=52$, p$=<0.10$). The null hypothesis was rejected for the slope estimate of the second week, $\hat{b}=-0.24\pm0.1$ ($t=2.4$, d.f.$=55$, p$=0.02$), and for that of the third week, $\hat{b}=1.04\pm0.25$ ($t=4.1$, d.f.$=55$, p$=<0.01$).

In spite of the variability of the data, in which some plants were severely affected by a relatively few aphid-days of attrack, and in which others were relatively unaffected by a comparatively large number of aphid-days of attack, there was a general tendency for lower plant weight to be associated with increases in aphid-days of feeding. Evidence for this association was most clearly demonstrated 1 or 2 weeks following release from infestation.

Presumably if the plants were allowed to continue to grow, at least three groups of plants could be selected: one composed of those very susceptible to injury, with little recovery potential, another in which there would be little or no evidence of attack, and an intermediate group. Whether or not the factor, or factors, associated with susceptibility to damage, and the factor, or factors, associated with recovery potential are genetically controlled, and if so, whether or not they are independent of one another, is not known.

Variations in aphid populations.—Maximum nymphal production (table 3) occurred on the fourth and fifth days after apterae were placed on the plants. In any one 24-hour exposure period, the single aptera produced a recorded total of from 0 to 10 nymphs, and the average daily nymphal production varied in experiments I and II, perhaps because of seasonal changes. The data given are not entirely accurate because of nymph losses, particularly during the latter portions of the 7-day infestation period. Since only losses, gains, or no change in the populations were tabulated daily, the apterae could have produced more progeny than were recorded.

Losses of nymphs during the exposure period probably reflected population pressure and host plant unsuitability. Unsuitability probably was due to inherent differences among plants as well as progressive changes induced by the insects. When populations were high, nymphs were restless and easily disturbed. The most severely damaged plants wilted badly, occasionally dropped leaves, and under these conditions, nymphs as well as apterae, tended to leave. The rate of nymph loss is given in table 4.

Movement and subsequent loss of apterae (table 4) took place rather spasmodically. Generally it occurred on the first or second day, or later under conditions of severe plant damage. In experiment II the evidence for bimodality was most clear. Initial loss perhaps was due to inherent host unsuitability. At times it was almost impossible to maintain single apterae on certain plants. In some cases the aptera would deposit a few nymphs before leaving, and in such cases the nymphs also tended to disperse. In other cases replacement of a missing aptera with another failed to stop wandering. Because of this, substitute plants were introduced into the finalized expermiments I and II.

Table 4.—Average[a] daily loss of spotted alfalfa aphids, both apterae and nymphs, during the 7-day infestation period of seedling alfalfa.

Day	Apterae		Nymphs	
	Experiment I	Experiment II	Experiment I	Experiment II
1	6.0	1.8	0.0	0.0
2	1.7	4.8	2.3	0.3
3	1.3	2.5	3.5	0.0
4	1.0	1.3	0.8	0.5
5	2.5	1.5	2.3	0.8
6	0.2	2.0	2.8	—
7	1.2	6.5	7.5	9.6

[a] Numbers in columns based on records on 240 plants in Experiment I, and 360 plants in Experiment II.

DISCUSSION.—One of the severest limitations to accuracy and precision of the experimental results was the variability encountered in host plants, insects, and the physical environment. It would be advantageous to be able to say, for example, that African alfalfa seedlings with a given aphid population, would within a specified time, react in a characteristic manner. Such was not possible. In some cases there were qualitative differences among individuals, *e.g.*, some failed to develop even the initial vein-banding symptom. In other cases the variations were more quantitative, *e.g.*, degree of stunting. Even without aphids, some plants failed to make appreciable growth. There were also large variations in the amount of recovery made during any one period of time. Severe gradations in temperature and moisture were encountered, and while partial compensation for this was provided by using randomized block designs, the within-treatment variation was still high. In spite of the use of the design, foundation seed, sterilized soil, and greenhouse conditions, the size of the experimental error left much to be desired.

Another persistent problem was the inconstant level of infestation. With the parthenogenetic aphids, numbers

Table 3.—Mean[a] (± standard deviation) daily nymphal production by single spotted alfalfa aphid apterae allowed to feed 7 days on healthy alfalfa seedlings.

Experiment	Day						
	1	2	3	4	5	6	7
I	1.6±1.7	1.6±1.7	1.6±1.7	1.9±2.1	2.0±2.0	1.2±1.7	1.3±2.0
II	2.1±1.0	2.8±1.7	2.9±1.6	3.2±1.8	3.3±1.8	2.9±1.9[b]	2.8±2.3[b]

[a] Experiment I used 240 apterae, Experiment II, 360 apterae.
[b] Based on means of three replications only.

could not be kept constant except for short periods by using nymphs. This is a relatively serious limitation when investigating a toxemia, since potentially each individual contributes to the totality of the disease effects.

There is much to learn of the assumed toxin. It would be desirable to isolate it in a relatively concentrated or pure form for purposes of identification. Conversations with H. T. Gordon, staff toxicologist, Department of Entomology and Parasitology, Berkeley, suggested that progress is dependent upon suitable bioassay methods. Crude experiments with juice inoculation of plants with extracts of macerated aphids failed to induce symptoms. It also was suggested that root growth suppression, using a technique similar to that used in the bioassays of certain phytotoxic chemicals, might have promise. Again only crude unsuccessful attempts have been made in this direction, using whole aphid extracts and germinating alfalfa seeds.

At present the limitations to direct study of the toxin would appear to be: 1) the small amount of toxin per aphid, 2) the lack of a suitable bioassay method, and 3) the lack of a method to introduce the toxin into susceptible tissues. Perhaps a glass-wick method would yield results, *e.g.*, one similar to that used by Beale & Jones (1951) for the successful introduction of certain antibiotics into living plants. Success would in part depend upon the *in vitro* stability of the toxin.

SUMMARY.—Greenhouse experiments on the effects of small populations (one aptera and progeny) of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton) feeding for 1 week on foundation African variety alfalfa seedlings (cotyledons plus the first monofoliate leaf) have indicated that the disease induced is a toxemia. The evidence for this is the inherent ability of the insects to induce the disease, the subsiding of symptoms following removal of the aphids, and the brevity of the incubation period.

Populations resulting from attempted initial infestation with one aptera varied from 0 to 35. Maximum nymphal production occurred 4 to 5 days after infestation. Maximum dispersal of aphids from infested plants occurred on the 7th day after infestation.

Individual plants varied both qualitatively and quantitatively in their reaction to infestation. However, it would appear that under the conditions tested, single aphids would not kill the plants, but that such populations as can result 7 days after infestation with a single aptera, could kill very susceptible individual plants of the African variety.

The initial symptoms, typically occurring within 48 hours of infestation, were yellow vein-banding. Later, local chlorotic areas on infested leaves, leaf drop, stunting, wilting, and necrosis developed in response to continued attack.

To measure stunting, total petiole length and fresh green weight were found comparable. The latter, being more feasible, was used.

Shell vial cages with various types of paper liners were found to result in reduced plant weight, and were eventually discontinued since the aphid had a strong tendency to remain *in situ*.

Infested and noninfested populations of seedlings could be statistically separated on the basis of fresh green weight 2 and 3 weeks after removal of aphids. Correlation between disease severity and number of aphid-days of attack was weak. However, the slope of the regression line was negative and statistically separable from zero.

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The Absorption and Degradation of DDT at Varied Dosages by the House Fly[1,2]

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To compare and to interpret the insecticidal action of different topical dosages of DDT, estimates of the amount of DDT absorbed are needed. Because acetone is a commonly employed solvent in insect toxicology, dosages of DDT in a constant volume of acetone were applied to house flies. Aborption and degradation of DDT were determined at 24 hours after treatment. Only data on survivors of DDT-resistant and susceptible strains of *Musca domestica* L. are reported.

Roan & Babers (1954) and Vinson & Kearns (1952) found an increase in absorbed DDT with increased dosages in *Periplaneta americana* (L.). The percentage absorption, however, was less as the applied dose increased. Similar results were also reported in *M. domestica* by Winteringham (1952), Perry (1952), and Tahori & Hoskins (1953). Percentages absorbed are not in agreement,

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Table 1.—Effect of varied topical dosages in 1.0 μl. of acetone on 6-day-old house flies held at 28.2° C. for 24 hours.

Per Cent DDT	Strain	Per Cent Alive	μg./Fly Dose[a]	μg./Fly in Survivors: DDT absorbed	DDE inside	DDT inside	NSH[b]	Per Cent of Dose: Absorbed	DDE inside	DDT inside	NSH	Per Cent of Absorbed as: DDE	DDT	NSH
0.01	CSMA	98	0.120	0.115	0.008	0.001	0.106	96	8	1	87	8	1	90
.02	CSMA	60	.190	.186	.026	.029	.131	98	14	15	69	14	16	70
.03	D+	98	.287	.276	.010	.010	.256	96	4	3	89	4	3	93
.04	D+	97	.398	.388	.017	.011	.360	98	4	3	86	4	3	93
.05	D+	99	.511	.503	.020	.008	.475	98	4	2	93	4	2	95
0.1	D+	90	.778	.734	.046	.007	.681	94	6	1	88	6	1	93
0.5	D+	99	4.42	2.86	0.96	0.13	1.77	65	22	3	40	34	5	62
2.5	D+	96	21.3	4.70	1.66	0.45	2.59	22	8	2	12	35	10	55
5.0	D+	95	44.0	10.1	1.66	0.66	7.80	23	4	2	18	17	7	77
10.0	D+	96	123.0	15.0	1.87	1.38	11.8	12	2	1	10	13	9	78
20.0	D+	91	212.0	36.4	1.59	2.78	32.0	17	1	1	15	4	8	88

[a] Verified by chemical analysis of freshly-treated, dead flies.
[b] Non-assayable by Schechter-Haller method.

probably because the conditions affecting absorption differ. Factors that influence absorption include time at assay (Perry, *et al.* 1953 and Sternburg *et al.* 1950), mortality (Lindquist *et al.*, 1951) site of application (Burtt 1945 and LeRoux & Morrison 1954), temperature (Barker 1957), choice of solvent (Barker & Rawhy 1957), volume of solvent (Roan & Babers 1954), and species (Buck & Posner 1949, Sarkaria & Patton 1949, Sternburg & Kearns 1952).

Ferguson & Kearns (1949) suggested that dosages of DDT, which exceeded the maximum that the insect can readily metabolize, will inhibit metabolism of DDT in *Oncopeltus fasciatus* (Dall.). *P. americana* given three dosages by Vinson & Kearns (1952) and by Roan & Babers (1954) continued to metabolize more DDT at the highest dosage. Neither could detect the principal degradation product by modified Schechter-Haller analyses.

PROCEDURE.—Several strains of house flies were reared by essentially the standard CSMA (Chemical Specialties Manufacturers Association) method (Anon. 1951). Pupae were placed in pint paper cans with screen lids. As the adults emerged in these cans they were fed diluted condensed milk. Six days after emergence, adults of mixed sexes were treated on the pronotum with DDT solutions from a screw-driven, 0.25-ml. tuberculin syringe. The treated flies were held at temperatures always within 2 degrees of those given in the data. Mortality was counted 24 hours after treatment. The smallest sample contained 20 flies. Between 20 and 500 suriving flies, depending upon the dosage, were selected for DDT analyses.

To measure the unabsorbed DDT, these flies were rinsed twice with 25 ml. of freshly distilled ether. The rinses were combined and analyzed for DDT by a modified Schechter-Haller method (Sternburg *et al.* 1950). Rinsed flies were pulverized in anhydrous sodium sulfate, and this brei was extracted with two 25-ml. portions of ether. This extract was analyzed for internal DDT and DDE [1,1-dichloro-2,2-bis (*p*-chlorophenyl)ethylene]. The unaccounted-for DDT is assumed to have been absorbed and is called "non-Schechter-Haller metabolite" (NSH).

To avoid dilution errors which could be confused with dosage effects, the dosages were verified by chemical analysis of freshly treated flies that had died of thirst. Applications to absorbent materials such as paper did not duplicate dosages to insects. The discrepancies between the expected concentrations of DDT and the detected dosages are obvious in table 1.

RESULTS AND DISCUSSION.—The absorptions at varied doses in 24 hours are given in figure 1. Generally, if dosages above 1 microgram per fly are changed, absorption is changed about half as much. The decrease in per cent absorption with increasing dosage is in close agreement with the rate reported by Perry (1952). Data of Sternburg & Kearns (1950) and Winteringham (1952)

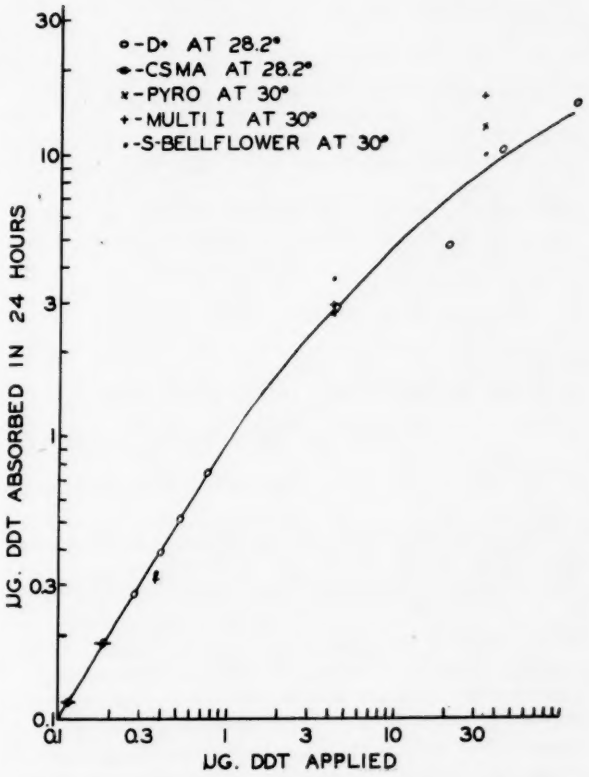

FIG. 1.—DDT absorbed by house flies with varied doses.

indicate a greater decrease in per cent absorption with increasing dosage. The amount of DDT that can contact a given area of cuticle is limited. Additional DDT increases the depth of the deposit, but it does not increase the DDT immediately available for absorption. The dosage in figure 1 was applied to strains D+ and CSMA in 1.0 μl. of acetone, and the per cent absorption decreases above application of 4 μg. Strains Pyro, Multi I, and s-Bellflower were treated with 1.5 μl., and the correlation between dosage and absorption is more linear. Although the susceptibility of the tested strains varies, the absorption of DDT in survivors shows no strain distinctions.

Surviving flies continue to form metabolites in increasing quantities with increasing dosages at dosages as high as 212 μg. per fly. The data in table 1 do not reveal a self-limiting constant which could suggest a cause of survival. Perhaps the DDT inside represents a gradient between large amounts of DDT on the cuticle and very small quantities at an unknown site of action.

When flies of the CSMA strain were treated with 0.190 μg., only 60% survived. These survivors degraded less of the absorbed DDT. This suggests that the inhibition of DDT degradation by excessive doses (Ferguson & Kearns 1949) is attributable to the toxicity of the DDT.

CONCLUSIONS.—Increased dosages at constant volume give increased absorption in surviving flies. However, at larger dosages the increases in absorption are proportionally less.

The amount of degraded DDT increases with higher dosages. Degradation varies from 84 to 99% of the absorbed DDT, but shows no trend dependent upon dosage. The survivors of several resistant strains give only indistinguishable differences in absorption.

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The Inhibitory Effect of Arsenic Trioxide on the Reduced Glutathione (GSH) of Several Tissues of *Periplaneta americana* (L.)[1,2]

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That arsenic and other heavy metals act on the reduced glutathione (GSH) of insects has been demonstrated (Fink 1926, Forgash 1951), but whether the lowering of the insect's GSH level is the prime cause of death is a moot question, for although there is evidence which suggests important functions of GSH, no definite role has been ascribed. It can be assumed, however, that any toxicant which reacts with the —SH group of glutathione will attack —SH groups in other body compounds if such groups are neither chemically nor physically shielded. —SH groups of certain proteins are known to be immune to attack by sulfhydryl reagents until they are denatured in such a way to expose the —SH groups, but there are many sulfhydryl compounds including enzymes which do not have this protection, and GSH obviously shields these vital substances against the action of sulfhydryl reagents. GSH contents of several insects have been

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reported (Fink 1926), but data on the concentration in individual tissues and organs, which may illucidate the function of GSH in insects, are not available. It also is desirable to know whether GSH in the various tissues is affected uniformly by *in vivo* treatment with arsenic.

To provide this information, the present study was conducted using the muscle, fat body, alimentary canal, and internal genitalia of the adult American cockroach, *Periplaneta americana*(L.).

MATERIALS AND METHODS.—*Determination of GSH.*—Reduced glutathione (GSH) was determined manometrically using the method of Woodward (1935) and Behrens (1941) with several modifications (Forgash 1951, 1956).

GSH preparations from insect tissues were made in the following manner. Male and female American cockroaches (which had been denied food and water for 24 hours) were anesthetized with CO_2, placed in a stoppered bottle, and allowed to recover from the effects of the gas. They were refrigerated then until ready for use. Immediately prior to dissection a small piece of gauze saturated with ether was placed in the bottle. In some tests decapitation of the cold-immobilized cockroaches was substituted for the ether treatment. Comparable results were obtained by both methods. Following dissection the tissues were rolled briefly in absorbent paper to remove adhering hemolymph, placed in tared vessels, and kept in the cold until all specimens (20 to 30) had been dissected. The weighed tissues were ground with 2% sulfosalicylic acid (2 ml./gm. of tissue) in an all-glass Tenbroeck homogenizer and stored under refrigeration overnight (16 to 18 hours) before conducting GSH determinations. Low amounts were obtained when the extraction period was reduced substantially. The suspension was filtered through a Buchner funnel and finally through a sintered-glass filter of fine porosity. GSH determinations were made on aliquots of tissue extracts prepared in this manner.

Injection of Arsenic Trioxide.—Measured quantities of solutions of arsenic trioxide (prepared by dissolving As_2O_3 in $NaHCO_3$ solution) were injected into the thoracic cavity by means of a foot-operated micro-injection apparatus, which is capable of delivering volumes as low as 1.00 ± 0.02 µl.

RESULTS.—*Distribution of GSH in the American cockroach.*—GSH determinations were conducted on the fat body, gonads, alimentary canal, and muscle of adult American cockroaches, using 20 to 30 insects for each test. The GSH values for the separate tissues are shown in table 1. The values are the average of three or four tests except in the case of male internal genitalia and male fat body where only one and two assays, respectively, were performed. In both sexes, the highest concentration of GSH was found in the alimentary canal, particularly in the mesenteron. The GSH content in all tissues tested ranged from 13% to 24% higher in the male than in the female.

The GSH concentrations of the tissues have been computed on a dry weight basis using the dry weight values shown in table 1. These values were obtained by drying to constant weight at 105° C. and represent the average of three measurements, each involving 20 to 30 insects.

Effect of Arsenic on the GSH of Tiwsues and Organs.—Male and female adult cockroaches were injected with solutions of arsenic trioxide. Five to six hours later a certain organ or a sample of a particular tissue was removed from 20 to 30 insects of each sex, and the reduction of GSH determined. In all tests, each tissue was assayed in triplicate and identical tests were conducted stimultaneously on similar tissues from untreated cockroaches. The results of these tests are shown in table 2.

The largest decreases in GSH following treatment with arsenic (Table 2) occur in the fat body and in muscle tissue. Since the greatest inhibitory effect occurs in the fat body, a direct comparison was made of the effect of arsenic on the total GSH of the entire insect and on the GSH of the fat body. Adult female roaches were injected with varying amounts of aresenic, and after $5\frac{1}{2}$ hours GSH measurements were made. Determinations were conducted simultaneously on untreated individuals and untreated fat body. The results are shown in table 3.

When the fat bodies of male and female cockroaches

Table 1.—The GSH content and dry weight of organs and tissues of adult male and female American cockroaches.

SEX	TISSUE OR ORGAN	DRY WEIGHT (Per Cent of Wet Weight)	GSH CONTENT µg./Mg. (Dry Weight)
Female	Muscle	25.2±0.3	1.36±0.18
Female	Mesenteron	25.8±0.2	3.32±0.44
Female	Alimentary Canal	28.3±0.5	2.66±0.24
Female	Fat Body	56.2±1.3	1.29±0.13
Female	Ovaries	44.1±1.5	0.67±0.05
Male	Muscle	25.8±0.1	1.55±0.08
Male	Mesenteron	24.2±0.3	3.90±0.46
Male	Alimentary Canal	29.4±0.6	3.28±0.35
Male	Fat Body	54.3±0.7	1.21±0.09
Male	Internal Genitalia[a]	31.1±0.9	1.09

[a] Sample consisted of testes, ducts, and accessory glands.

Table 2.—The effect of As_2O_3 on the GSH of tissues and organs of adult male and female American cockroaches.

TISSUE OR ORGAN	SEX	As^{+3} TREATMENT (µMOLS./GM. INSECT)	PER CENT INHIBITION OF GSH
Alimentary Canal	Male	0.101	20
Alimentary Canal	Male	.155	5
Alimentary Canal	Female	.135	9
Alimentary Canal	Female	.176	11
Fat Body	Female	.162	61
Muscle	Male	.152	30
Muscle	Female	.165	25

Table 3.—Comparison of the effects of As_2O_3 on the GSH of the fat body and of the entire female adult American cockroach.

SAMPLE	As^{+3} TREATMENT (µMOLS./GM. INSECT)	PER CENT INHIBITION OF GSH
Fat Body	0.158	56
Entire insect	.152	18
Fat Body	.183	80
Entire insect	.181	26

were removed and weighed, the wet weights for females in separate tests were 148, 137, 105, and 94 mg. per insect (average = 124 mg.) and for males, 17 and 16 mg. per insect. Although it was not possible to extricate the fat body entirely from the surrounding organs and tissues, it was estimated that only a fraction of the total content remained after dissection. Before placing the fat body in corked vials, it was rolled on absorbent tissue to remove adhering hemolymph. Tissues from 9 to 11 insects were used for each measurement.

DISCUSSION.—The female of the species is, as a general rule, less susceptible than the male to a given insecticide. This is true of the American cockroach in relation to the toxicity of arsenic, the female exhibiting considerably greater tolerance (corrected for body weight) than the male (Heal 1942, Forgash 1956). The reasons for this difference in susceptibility are obscure. It is known, however, that arsenic will react with the GSH in the tissues, and since it has been shown that injections of GSH will protect against the toxic effects of arsenic (Forgash 1951), the combination between arsenic and body GSH can be assumed to reduce the amount of toxic arsenic available for reaction with other substances in the tissues. If the inactivation of a certain portion of GSH does not constitute a real hazard to the insect, then GSH apparently performs the function of a detoxifying agent. Thus, any insect which has an available supply of GSH greater than another insect has a potentially greater tolerance to arsenic.

Since in the American cockroach, GSH concentrations of several organs and tissues range from 13% to 24% higher in the male than in the female, the male tissues can be said to be better fortified against the action of arsenicals. After injection with arsenic, all tissues tested showed a decrease in GSH, but the most pronounced effect occurred in the fat body, indicating that arsenic is "picked-up" by these tissues. Assuming that the action of arsenic on the fat body does not contribute measurably to the death of the insect, here may be a logical explanation of the higher tolerance of the female. The GSH concentration of the fat body of the female cockroach is 1.29 μg./mg. of dry weight (table 1) and since the female has 60 mg. (dry weight) more fat body than the male there is more GSH to react with arsenic or other sulfhydryl inhibitors. The LD_{50} dosage of injected As^{+3} is three to four μg. higher for the female than for the male cockroach. The chemistry of the reactants indicates that 3 moles of GSH combine with one mole of trivalent arsenic (Voegtlin *et al.* 1923), and thus the excess of GSH in the female fat body (77.4 μg. or 0.256 μmols.) is capable of reacting with 0.085 μmols. or 6.4 μg. of trivalent arsenic, which is considerably more than the difference between the LD_{50}s for the two sexes. This is especially noteworthy when we consider that the fat body, ramifying freely throughout the entire coelom, enveloping the muscles and organs of the thorax and abdomen, is in an ideal position to contact any arsenic which enters the blood stream.

In addition to the excess fat body there is more than three times as much gonadial tissue in the female as in the male, and, although the GSH concentration of these tissues is lower than that of the fat body, there is an appreciable difference between the two sexes with respect to the total quantity of GSH in the gonads.

The method used to report the GSH content of tissues can be misleading in some instances where comparisons are made between certain tissues. For example, the fact that in the female cockroach the GSH content of the fat body is 1.29 μg./mg. of dry tissue weight and that of the muscle is 1.36 might lead to the conclusion that the GSH pattern is essentially the same in the two tissues, but, the dry weights of muscle tissue and fat body are approximately 25% and 56%, respectively, and most of the difference is due probably to the presence of water and larger amounts of lipids in the "fat" tissue. If, therefore, the GSH concentration of the fat body were computed on a basis exclusive of fats and urates, a much higher value would be obtained.

SUMMARY AND CONCLUSIONS—A study was made of the effects of As^{+3} on reduced glutathione (GSH) in the muscle, fat body, internal genitalia, and alimentary canal of the adult American cockroach. The GSH concentration in different tissues varied considerably, the highest concentrations (based on total dry weight) appeared in the alimentary canal, particularly in the mesenteron. The concentration in both muscle tissue and fat body was approximately 50% of the amount found in the mesenteron. The internal genitalia contain smaller quantities. The greatest decreases in GSH following injection with arsenic occurred in the muscle and fat body, with the reduction in the latter being the more pronounced. This coupled with the fact that the female contains approximately eight times as much fat body as the male may explain the higher tolerance of the female to arsenic.

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Biotic Factors in Natural Control of Citrus Mealybugs in California[1]

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The problem of the citrus mealybug, *Pseudococcus citri* (Risso), on citrus trees in Ventura County, one of the largest citrus districts of California, has become acute in recent years. Increased damage has occurred despite greater efforts toward ant control. In a number of cases the intensity of infestation appears to be associated with insecticidal drift to citrus orchards from nearby DDT-treated walnut and vegetable plantings. The increased damage in some instances may also be related to the current decreased reliance of growers upon oil sprays.

In the case of DDT drift from adjoining treated acreages, a gradient increase in the mealybug population is clearly defined with proximity to the DDT-treated source. In the case of the inverse correlation with oil spray treatments, the shift in recent years from maintenance oil sprays to emergency acaricide treatment has in certain instances been attended by greater overall mealybug damage. It did not appear logical to associate this increase with the discontinuance of oils, since oil sprays are very inefficient mealybug insecticides and maintenance oil treatments are generally applied at a seasonal period when mealybugs are at their lowest density. However, the supposition that the new organic acaricides were in themselves causing a mealybug upset was shown to be inconsistent with a series of laboratory tests (unpublished data) that showed these suspected materials innocuous to a number of the most important species of mealybug parasites and predators.

Six species of predators and four species of parasites are at times associated with *Pseudococcus citri* in the Ventura County area studied. The predators are *Cryptolaemus montrouzieri* Muls.; *Hyperaspis lateralis* Muls.; *Leucopis bella* Loew., *Scymnus sordidus* Horn; the brown lacewing, *Sympherobius californicus* Banks; and the green lacewing, *Chrysopa plorabunda* Fitch (*californica* Coq.). The parasites represented were *Leptomastidea abnormis* (Gir.), *Pauridea peregrina* Timb., *Leptomastix dactyloppi* How., and *Allotropa citri* Mues.

In order to integrate insecticidal pest control practices with natural control of citrus mealybugs it was considered necessary, first, to assess the seasonal effectiveness of all the natural enemies under conditions of minimum upset—that is, where the host-natural enemy balance was least disturbed. Evaluating the effectiveness of the various natural enemies at low host densities was indicated as being particularly necessary, since oil application at this low density period seemed to retard mealybug increase.

Procedure.—The seasonal assessment of natural enemy effectiveness at both low and high host densities was impossible with existing techniques. On the basis of previous experience with insecticidal trap areas (DeBach & Bartlett 1951), a new check method was devised to obtain the required data. This method consisted essentially of an untreated series of trapped trees surrounded by an insecticidally treated barricade area of trees. Periodic population counts of hosts and natural enemies in the untreated trapped area could then be compared with similar population counts in an outside check area to indicate the host population change in response to seasonal natural enemy activity.

For this experiment a 3-acre Ventura County Valencia orange grove was used, in which there was practically an undisturbed state of balance between natural enemies and citrus mealybugs. The grove had remained virtually undistributed, so far as mealybugs were concerned, for the preceding 3 years, only one oil-rotenone treatment and two citrus red mite treatments having been applied during that time, in addition to maintenance deficiency, and plant hormone sprays. Ants had been successfully kept out of the grove by ground treatment with chlordane.

In the center of this 3-acre block, four adjacent trees in a single row were chosen for the untreated, trapped area. The two rows of trees immediately surrounding the four central untreated trees were treated on July 12, 1955, with heavy dosages of DDT-kerosene. The treated trees acted both as a barrier against immigration of natural enemies from outside and as a trap for the natural enemies on the four central trees, eventually destroying them as they moved from the four central trees to the treated barricade area. The DDT-kerosene dosage consisted of 1.5 lbs. technical DDT dissolved in 3 gallons of kerosene per 100 gallons of water, applied with very thorough coverage. Fumigation tents over the four central test trees at treatment time protected them from drift contamination.

To prevent increase of the citrus red mite to the damaging proportions that so often attend heavy DDT treatment on citrus, the DDT-treated barricade trees were also treated with trunk applications of demeton (Jeppson *et al.* 1954). In the course of 13 months' conduct of this test, periodic bioassays were made of the toxic residue remaining on the treated barricade trees. Only one additional DDT application was made, on February 22, 1956, using 1.5 lbs. 50% DDT as wettable powder, plus 1 pint summer spreader per 100 gallons of water. This treatment sufficed for 10 months. By that time DDT residues had become fairly low, but since the experiment was nearing completion no further treatment was made. The first tree-trunk application of demeton was highly successful. The second comparable treatment, on February 8, 1956, was not effective, however, and fairly high citrus red mite populations finally caused some tree injury.

Thirteen consecutive monthly counts were taken of the populations of citrus mealybug and its natural enemies during the experiment. The count areas selected were the following: (1) the four untreated central trapped trees; (2) another four DDT-treated and demeton-treated trees, each tree of which cornered on the four central trapped trees; and (3) four untreated check trees, each tree of which was six rows distant from the corners of the treated area. Counts also were taken on the four outside corner trees of the treated area, where no mite control (demeton trunk treatment) was ever made; but since the counts

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were identical with those on trees which received demeton no use was made of those data. It was of interest, however, that damaging populations of citrus red mite developed on these four trees and ultimately spread to the ajoining untreated areas to cause an obvious upset throughout the grove. Mealybug counts consisted of the total number of citrus mealybugs encountered on the four count trees in each plot during a 10-minute search per tree.

Natural enemy counts consisted of a combination of (1) natural enemies observed during the 10-minute tree counts, (2) parasites or predators emerging from a mealybug sample brought to the laboratory for counting, and (3) predators captured by removal of a burlap band surrounding the trunk of each count tree. These natural enemy counts, particularly those of the 10-minute tree search, gave a good quantitative census of predator activity. However, the rearing samples were sometimes inadequate for other than a qualitative assessment of parasite activity.

To interpret the test results properly, it is necessary first to recognize the pattern of DDT toxicity to the citrus mealybug and the various natural enemies. DDT is practically innocuous to all forms of the citrus mealybug except the newly hatched stage, against which it is fairly effective. The parasite *Leptomastidea abnormis* is extremely sensitive to DDT. *Chrysopa* larvae are very tolerant to DDT. There was no evidence throughout the experiment of any restriction in the activity of this predator by DDT. Tolerance of *Cryptolaemus montrouzieri*, *Coccinella californica* Mann., and *Hyperaspis lateralis* to DDT increased in the order named, *Cryptolaemus* being fairly susceptible, *Coccinella* somewhat tolerant, and *Hyperaspis* the most tolerant. The DDT-tolerance pattern seems to apply similarly to both adult and larval forms of these coccinellids. Although very few larvae of *Chrysopa plorabunda* were observed in the counts, there is reliable evidence that larvae of this species are tolerant to DDT (Doutt & Hagen 1950).

In this test the DDT-treated barricade area did not completely exclude the predators from the central untreated area. *Chrysopa* adults were not excluded. *Coccinella* adults and *Cryptolaemus* were well excluded until May, when the 2-tree-row DDT-treated barricade was no longer completely effective. Exclusion of the parasite *Leptomastidea* from the untreated central area was completed very rapidly, and 4 months after treatment this species was even eliminated from the outside check trees.

The periodic counts for citrus mealybug population changes in the three differently handled plots are presented in graphic form in figure 1. The curve for the DDT-demeton-treated plot was virtually identical with that for the DDT-no-demeton-treated plot, so only the curve for the former is shown on the chart. No attempt has been made to present graphically the extensive natural enemy data obtained in the course of this experiment. Where these data are pertinent to an explanation of the mealybug population changes, the significant trends are discussed in the text. The initial infestation of citrus mealybug in July, 1955, was very low. The population consisted of occasional very small mealybugs under the fruit buttons, and of rare, nearly full-grown mealybugs in the off-bloom fruit clusters.

CITRUS MEALYBUG POPULATIONS IN THE ABSENCE OF NATURAL ENEMIES.—Without the intervention of natural

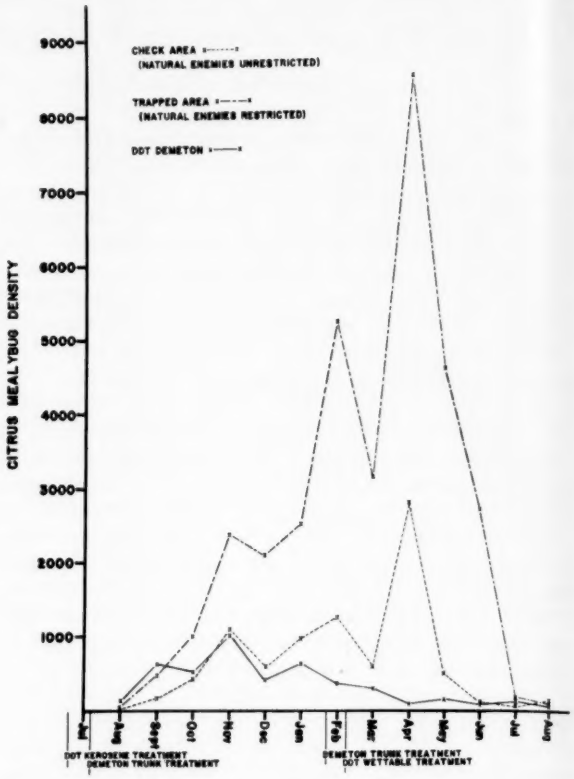

FIG. 1.—Periodic counts of populations of the citrus mealybug, *Pseudococcus citri* (Risso), in three Valencia orange plots in Ventura County, 1955 to 1956, demonstrating the effect of restricting or eliminating species of natural enemies.

enemies or other control methods, citrus mealybug populations in the area of this test tend to remain at a high level all year round. In this experiment, except for the last 3-month period of the test, when natural enemies finally broke through the DDT barricade, the normal population is best represented by the curve for the trapped area (fig. 1). The green lacewings were not eliminated from this area, so their influence cannot be isolated. From the population curves for all the plots counted, it would appear that four to five broods of mealybugs occur annually in the field.

The minor deflections from a steady increase in the mealybug population from July to the following April are associated with both the responses of the mealybugs to climate and the activity of green lacewings. In the test there was no way to separate these two influences. The major decline in citrus mealybug populations from February to March was associated with a severe cold period in Late February.

THE DIRECT EFFECT OF DDT ON CITRUS MEALYBUGS.—The effect of DDT on citrus mealybugs can be isolated by comparing the population curve for the DDT-demeton-treated area (fig. 1) with that for the trapped area. All relative decreases in population density shown on the former curve subsequent to September were associated with the effect of DDT on newly hatched mealybugs. This continued decrease, in comparison with the curve for the trapped area, could not be other than the quantitative effect of DDT on the mealybugs.

Whether this response was due entirely to a direct effect of the DDT on the mealybugs, or whether the DDT was acting indirectly through the tree, is indeterminable.

The initial increase to a September peak in the DDT-treated plots represents the rapid response of mealybugs to the immediate destruction of natural enemies and is discussed in detail below. The populations on the DDT-treated trees did not at any time reach a density which in commercial practice would have required auxiliary chemical pest control treatments. On this graph any population density exceeding the 1,000 mark might ordinarily be considered severe enough to warrant chemical treatment.

THE EFFECT OF NATURAL ENEMIES ON CITRUS MEALYBUGS.—The total effective influence of natural enemies on citrus mealybugs is represented in this experiment by differences between the outside check area, where natural enemies were present, and the central untreated (trapped) area, where natural enemies were restricted. The difference in areas subtended by the two curves (fig. 1) shows that the natural enemies were responsible in this test for a 75% reduction in mealybug population. This amount of mealybug reduction occurred despite the fact that none of the biological control due to green lacewings and only that part of the coccinellid effect occurring before April was considered. Furthermore, only the very early *Leptomastidea* activity was included.

The outstanding reduction in the citrus mealybug populations occurred in relation to the peak period of *Cryptolaemus* activity. The major activity of this predator started in late April and declined in June as the mealybug population declined. A previous very light density of *Cryptolaemus* in November and December played no part in suppressing mealybug abundance, since no *Cryptolaemus* were found at that period in either the DDT-treated or the trapped areas, where the mealybug populations also declined. This was most likely the restraint imposed by the first cold weather upon the citrus mealybugs. *Cryptolaemus* is ordinarily reared in commercial insectaries in the Ventura County area and periodically released in mealybug-infested orchards. Although no releases were made in this test orchard in the course of the experiment, it is possible that the *Cryptolaemus* influx to the orchard did not exclusively represent naturally occurring populations.

It is of interest that a natural influx of adult *Coccinella californica* during April preceded the activity period of *Cryptolaemus* in this test orchard. This species started the mealybug decline, but within a period of a month the limited efficiency of *Coccinella* was overshadowed by that of the more efficient *Cryptolaemus*. Aside from the *Chrysopa* effectiveness, which was not measured in this test, little or no mealybug reduction could be associated with the action of the other predators, including *Leucopis*, *Hyperaspis*, *Sympherobius*, or the extremely rare *Scymnus sordidus*. *Chrysopa* was equally abundant in the outside check and the untreated central trapped aeas, indicating no restriction on egress or ingress.

The activity of *Leptomastidea* was correlated in these tests with an interesting example of restraint upon mealybug increase. Even at the time of the first complete counts, in August, 1955, the result of differential destruction of *Leptomastidea* was evident between the outside check area and the other areas where this species was suppressed. The rapid destruction of *Leptomastidea* in the DDT-treated plots was most quickly reflected in a mealybug increase, despite the conflicting direct effect of DDT in destroying newly hatched forms. The rapid and complete elimination of this species, even from the outside check area, by November, 1955, permits the seasonal effect of this species to be evaluated. Until elimination of *Leptomastidea* was finally completed it was the only species that could be found working on the very small individual mealybugs under the fruit buttons, except for the few *Chrysopa* larvae which were equally represented in both areas. The entire difference between the November mealybug populations in the outside check area and in the central trapped area is believed to be related principally to the action of this species. *Leptomastidea* at this season may be of considerable importance as a restraint on incipient mealybug increase.

It was of interest that although *Leptomastidea* continued its activity uninterruptedly, where followed in three other citrus orchards in the vicinity, no large seasonal population fluctuations of the parasites were noted. It would by inference appear that its greatest influence in the area studied may be concentrated at the period of generally lowest host density during the late summer and fall. Any possible retardation of incipient outbreaks of *Pseudococcus citri* by *Leptomastidea* could easily be upset by insecticide treatments at that time, if the material used were inimical to the parasite.

SUMMARY.—The citrus mealybug, *Pseudococcus citri* (Risso), has become more severe in some citrus districts in southern California in recent years. Localized outbreaks of citrus mealybug frequently are associated with the destruction of natural enemies by DDT drift from adjoining cultivated crop areas. A seasonal evaluation of the effectiveness of the natural enemies of citrus mealybugs was made by associating their natural abundance with the increase and decline of mealybug populations in three areas, one where natural enemies remained undisturbed, a second area where certain natural enemies were restricted by surrounding the untreated plot with a barricade of DDT-treated trees, and a third area where natural enemies were restricted by direct application of DDT to the trees. From the seasonal mealybug population changes in the three plots and the natural enemy associations, an assessment of parasite and predator effectiveness has been isolated from abiotic factors including the effects of DDT on the host population. *Cryptolaemus montrouzieri* Muls. was found to be a very effective predator at high host densities. In this experiment the parasite *Leptomastidea abnormis* (Gir.) was associated with citrus mealybug population decrease at periods of low host density. The effectiveness of the green lacewing, *Chrysopa plorabunda* Fitch, could not be evaluated with the methods employed.

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Control of the Codling Moth and Other Apple Pests with Ryania[1]

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The insecticide ryania consists of the ground stems of *Ryania speciosa*, a shrub or small tree native to Trinidad. The results of tests for control of the codling moth (*Carpocapsa pomonella* (L.)) in 1945 and of additional tests for control of this and other apple pests from 1953 to 1956 are reported in this paper.

In 1945 ryania, 6 pounds, was compared with DDT, 1 pound, and lead arsenate, 3 pounds, per 100 gallons of spray in replicated field plots in eastern New York. There were 96 worms and 119 total injuries (worms and stings) per 100 apples on the unsprayed check trees. Percentage reductions in worms per 100 apples were 99, 95, and 74 for DDT, ryania, and lead arsenate, respectively, and in total injuries per 100 apples 95, 50, and 9. Although ryania was definitely superior to lead arsenate, it was inferior to DDT, and experimental work with it was discontinued.

In 1953 investigations with ryania were resumed in Indiana, soon after there were indications in some orchards that DDT was not always effective. An activated product containing 95.8% of ryania and 3.2% of propyl isome was used from 1953 through 1955 and 100% ryania in 1956.

Table 1.—Comparative effectiveness of activated ryania and DDT against codling moth larvae in field-laboratory tests.

Materials per 100 Gallons	Age of Deposit (Days)	Per Cent Reduction in— Entrances	Injuries
1953			
Ryania			
6 lb.	0	88	86
	7	89	89
6 lb. plus summer oil 2 qt.	0	90	90
	7	87	86
DDT (50%) 2 lb.	0	93	93
	7	87	86
Unsprayed check (per cent making entries)	0	65	63
	7	60	75
1954			
Ryania			
6 lb.	0	97	97
4 lb.	14	58	56
4 lb. plus summer oil 2 qt. and nicotine bentonite (14%) 2 lb.	0	97	97
	14	93	91
DDT (50%) 2 lb.	0	99	97
	14	90	91
Unsprayed check (per cent making entries)	0	34	77
	14	37	89
1955			
Ryania			
6 lb.	0	99	99
3 lb.	9	90	39
3 lb. plus summer oil 2 qt. and nicotine sulfate ½ pt. plus Mississippi bentonite 1 lb.	0	99	97
	9	98	75
DDT (50%) 2 lb.	0	94	92
	9	73	68
Unsprayed check (per cent making entries)	0	67	74
	9	61	76

In 1953 activated ryania at 6 pounds per 100 gallons was used, alone and with 2 quarts of summer oil, in comparison with 50% DDT at 2 pounds of a wettable powder per 100 gallons. In 1954 and 1955 the samples for laboratory infesting were taken from trees in the small replicated field plots. From 40 to 60 apples per treatment, picked at random, were infested with 10 codling moth larvae for each test. In 1953 the fruit received only one application, but in 1954 and 1955 the samples were taken after the sixth cover spray, affording an opportunity for residues to increase as the season progressed.

The treatments and results are given in table 1. Ryania compared favorably with DDT, especially when supplemented with oil or oil plus nicotine. The most noticeable difference between treatments was the lower reduction in injuries by weathered deposits of ryania in 1954 and 1955 as compared with those by DDT or ryania plus nicotine bentonite and oil. The need for using a sticker with ryania was indicated and that, if a sticker were used, reduced amounts of ryania might give satisfactory control.

Laboratory Tests.—Evaluation of a codling moth insecticide is incomplete without a determination of its effect on adults and eggs as well as the larvae. Toxicity to these stages is a valuable asset of any insecticide. Parathion and malathion are good examples of insecticides having this attribute. Tests in 1955 showed that ryania is not particularly effective against these stages. At 5 pounds per 100 gallons 28 or 33% of the adults were killed in 24 hours, depending on whether they were sprayed or left to crawl over heavily sprayed surfaces for 1 hour. Under the same conditions 2 pounds of 50% DDT wettable powder per 100 gallons killed 13 and 66%, 1 pound of 15% parathion wettable powder 80 and 100%, and water alone 8 and 28%. When eggs up to 5 days old were exposed to these insecticides, 46% were killed by ryania, 15% by DDT, 99% by parathion, and 16% by water.

Small-Plot Field Tests. *Codling Moth Control.*—Ryania alone and in combination with nicotine and/or oil was compared with DDT for codling moth control in single-tree plots, replicated three to six times, from 1954 through 1956. Captan was included when a fungicide was

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needed, except that zineb was used in sprays containing oil. From seven to nine applications were made each season, depending upon the severity of attack by third-brood worms. The first application was made at the time of either the first or second cover spray, about 10 or 20 days after petal fall, depending upon early moth activity. High-pressure sprayers equipped with single-nozzle guns were used. From 20 to 25 gallons were applied to each tree. Treatments in 1954 and 1955 were the same as given in table 1; in 1956 ryania alone at 6 pounds and 50% DDT wettable powder at 2 pounds per 100 gallons were compared with 3 pounds of ryania plus ½ pint of nicotine sulfate, and, in the first four cover sprays, 1 pound of Mississippi bentonite.

The results of these tests are shown in figure 1. In 1956 the codling moth infestation was light, and all treatments gave excellent control. In 1954 and 1955, when the infestation was heavier, ryania was better than DDT in preventing worm holes but was much less effective in preventing stings. The presence of a few stings is not a serious matter, but the number on the ryania-sprayed fruit was large enough to be objectionable. In both these years the ryania-nicotine-oil combination was more effective than either ryania or DDT alone in preventing both worm holes and stings. Unfortunately, there are two serious objections to the use of oil; it is incompatible with some of the common fungicides, and it may affect fruit finish adversely. Therefore, it was omitted from the combination spray in 1956. This omission resulted in a greater number of stings in the ryania-nicotine plot than in the DDT plot, whereas in 1954 and 1955, when oil was included in the combination spray, there were more in the DDT plots.

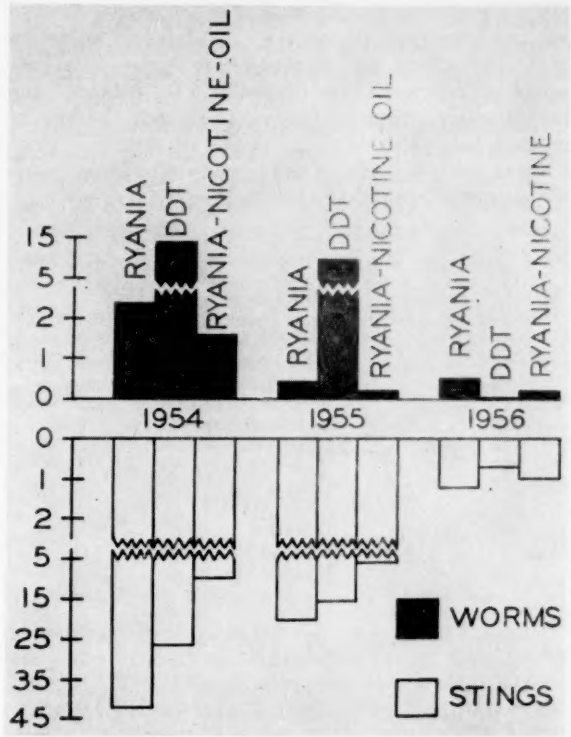

FIG. 1.—Codling moth injuries per 100 apples in small-plot field tests in Indiana.

Control of Other Apple Pests.—Observations in the field plots in 1955 indicated that ryania could not be depended upon to control some of the other apple pests. In the ryania plot 22.4% of the fruit was injured by the plum curculio (*Conotrachelus nenuphar* (Hbst.)), 10.2% by Forbes scale (*Aspidiotus forbesi* Johns.), and 4.9% by the red-banded leaf roller (*Argyrotaenia velutinana* (Wlk.)). In the DDT plot the respective percentages were 23.2, 16.2, and 5.8, and in the DDT-parathion plot 2.6, 0, and 0.3. Use of nicotine and oil with ryania prevented infestation by Forbes scale, but had no effect on the injury caused by the red-banded leaf roller. At various times heavy infestations of mites developed where ryania was being applied to small plots, and mite injury was as serious as where DDT was used. Nicotine bentonite, alone and with oil, when included with ryania tended to keep the number of mites at a low level. It was evident that ryania by itself is not an effective acaricide and that its substitution for DDT, at least for the treatment of small plots, is no guarantee that mites will not become a problem.

TESTS IN LARGE FIELD PLOTS.—In 1956 growers cooperated in two large-scale tests comparing ryania with their standard sprays. One test was in a 20-acre block of 6-year-old trees that had just begun to bear. These trees were selected because they had not received repeated applications of spray chemicals known to be destructive to beneficial insects. The second test was in a 10-acre block of mature trees that had been poorly pruned. The grower had applied DDT to this block for about 10 years, and supplemented it with parathion for the last 5 years. Populations of the European red mite, *Panonychus*(=*Metatetranychus*) *ulmi* (Koch), the red-banded leaf roller, and the codling moth had been high for several years.

A sulfur dust and spray program for disease control was used on all trees by both growers in prebloom applications. The insecticide program in the young orchard was as follows (quantities per 100 gallons):

Date of Application	*Ryania program*	*Grower program*
March 6 (dormant)	Oil 3 gal.+dinitrocresol 1 lb.	
May 4 (petal fall)	Lead arsenate 3 lb.	Lead arsenate 3 lb.+demeton (25%) 6.2 oz.
16	Lead arsenate 3 lb.	Lead arsenate 3 lb.
28	Lead arsenate 3 lb.+ryania 6 lb.	DDT (50%) 1½ lb.+TDE (50%) 1½ lb.+malathion (25%) 2 lbs.
June 8	Lead arsenate 3 lb.+ryania 6 lb.	DDT (50%) 1½ lb.+malathion (25%) 2 lb.
20	Ryania 6 lb.	DDT (50%) 1½ lb.+malathion (25%) 2 lb.
July 3–6	Ryania 6 lb.	DDT (50%) 1½ lb.+malathion (25%) 1¼ lb.
28	Ryania 6 lb.	DDT (50%) 1½ lb.+demeton (25%) 6 oz.
Aug. 16 (south side of trees)	Ryania 6 lb.	Malathion (25%) 2 lb.
24 (north side of trees)	Ryania 6 lb.	Malathion (25%) 2 lb.

In the ryania program captan, 1–2 pounds per 100 gallons, was the fungicide in the petal-fall and all subsequent applications, and in the grower's program sulfur-ferbam, 3 pounds, was the fungicide in the petal-fall and first four cover sprays and captan, 1 pound, in the remaining applications.

The insecticide program in the mature orchard was as follows (quantities per 100 gallons):

Date of application	*Ryania program*	*Grower program*
March 18 (dormant)	Oil 3%+dinitrocresol 1 lb.	
May 2–8 (petal-fall)	Lead arsenate 3 lb.+TDE (50%) 1½ lb.	Lead arsenate 3 lb.+TDE (50%) 1½ lb.+demeton (26%) ½ pt.
17	Lead arsenate 3 lb.+ovex (40%) ½ lb.	Lead arsenate 2 lb.+DDT (50%) 2 lb.
28	Lead arsenate 3 lb.+ryania 6 lb.	Lead arsenate 2 lb.+DDT (50%) 2 lb.
June 6	Lead arsenate 1½ lb.+ryania 6 lb.	DDT (50%) 1½ lb.+parathion (15%) ½ lb.
16	Ryania 6 lb.+ovex (40%) ½ lb.	DDT (50%) 1½ lb.+parathion (15%) 1 lb.
18 (top-off)	Ryania 6 lb.+ovex (40%) ½ lb.	DDT (50%) 1½ lb.+parathion (15%) 1 lb.
25	Ryania 6 lb.+ovex (40%) ½ lb.	DDT (50%) 1½ lb.+parathion (15%) ½ lb.
July 5	Ryania 6 lb.	DDT (50%) 1½ lb.+parathion (15%) ½ lb. +TDE(50%) 1½ lb.
20	Ryania 6 lb.	Ryania 6 lb.+demeton (26%) ½ pt.
Aug. 3	Ryania 6 lb.	Ryania 6 lb.
16	Ryania 6 lb.	Ryania 6 lb.

Captan, 1 to 2 pounds per 100 gallons, was included aa the fungicide in the petal-fall, first through third and fifth through eighth covers in both programs except the grower substituted ferbam, 1 pound, for the captan in the petal-fall application to trees of the Rome variety. No fungicide was included in the remaining applications.

The results of these tests are given in table 2.

In the ryania program good control of the apple insects in the young orchard, with the help of a dormant oil-dinitrocresol spray, kept orchard mites under control. However, the grower's DDT-malathion program did even better than the ryania program, particularly in control of the codling moth and plum curculio. Other insects presented no problems in control in either plot.

Insect and mite control in the mature orchard was handicapped by poor spray coverage, and neither program gave entirely satisfactory results. The ryania performed poorly on the Turley variety, and the grower's program (DDT-parathion through the main part of the season and ryania at the end) performed poorly on the Grimes and only moderately well on the Rome variety. Because of the poor coverage, differences in control between treatments are not considered significant. Populations of the leafhopper, *Erythroneura lawsoniana* Bak., built up to a high level in the ryania block during July and spread into the check area. No control measures were applied, and a moderate degree of leaf mottling occurred. European red mites also started to build up in this block on two occasions, and ovex sprays were applied to reduce them. The indications in this orchard were that the natural enemies of mites were at such a low level because of the previous exposure to insecticides that it would take more than one season for them to return to an effective level.

Rotenone-pyrethrum sprays were applied to sample trees in the ryania and check plots in both orchards to knock down the insects present. The identification of the species collected is incomplete, but has progressed far enough to show that the insect fauna in the ryania plots differed markedly from that in the DDT-malathion or

Table 2.—Comparative effectiveness of ryania and grower spray programs in controlling insects and mites attacking apples, 1956.

Spray Program	Variety	Per Cent of Clean Fruit	Number of Codling Moth Injuries per 100 Apples		Per Cent of Fruit Injured by—			Number of European Red Mites and Eggs per 100 Leaves			
			Worms	Stings	Red-banded Leaf Roller	Plum Curculio	Forbes Scale	June 12–13	June 26	July 11	July 23–24
					Young orchard						
Ryania	Golden Delicious	94	0.5	2.0	0.7	2.8	—	0	—	27	63
	Jonathan	93	0.6	3.8	.3	2.6	—	1	—	7	87
	Turley	96	1.0	3.0	.2	.3	—	0	—	33	236
Grower (DDT-malathion)	Golden Delicious	99	0	0.5	0.2	0.3	—	0	—	0	0
	Jonathan	99	0	.8	.5	0	—	0	—	1	.0
	Turley	97	0	.5	0	1.3	—	0	—	0	0
					Mature orchard						
Ryania	Grimes	90	4	5	1.3	0.8	0.8	832	712	—	139
	Turley	64	15	27	3.4	1.4	2.3	258	564	—	1696
Grower (DDT-parathion)	Grimes	75	14	9	2.0	1.2	4.5	6	924	—	262
	Rome	77	9	7	6.2	3.4	0.8	17	56	—	690

DDT-parathion plots. It is likely that final determinations will show that the larger number of beneficial forms was present in the ryania-sprayed plots.

SUMMARY.—In field and field-laboratory studies in New York and Indiana ryania gave control of the codling moth (*Carpocapsa pomonella* (L.)) when used at 6 pounds per 100 gallons of spray and compared favorably with DDT. Use of nicotine-oil materials with 3 to 4 pounds of ryania gave improved results, particularly in reducing the number of stings permitted by ryania alone.

Ryania acted primarily against the larval stage of the codling moth. Its effect on the egg and adult stages was low in comparison with parathion and malathion, but compared fairly well with DDT.

A ryania program gave good control of other apple pests in a young orchard and, with the help of a dormant oil-dinitrocresol spray, kept orchard mites under control. The check DDT-malathion program was even more effective, however.

Under conditions of poor spray coverage neither a ryania nor a DDT-parathion program gave satisfactory control of insects in a mature apple orchard. Most differences in control were not significant. However, in the ryania block the leafhopper, *Erythroneura lawsoniana* Bak., built up to high proportions and two applications of ovex were required to check increases of the European red mite, *Panonychus* (= *Metatetranychus*) *ulmi* (Koch).

Incomplete data indicate that beneficial species were more abundant in the ryania plots than in the DDT-parathion or DDT-malathion plots.

Experiments on Dimorphism in the Female Honey Bee[1]

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Dimorphism in the female honey bee is known to be controlled by the nutrition of the larvae, but the mechanism of the control has not been well elucidated. The factors that cause nurse bees to give a larva the food that is appropriate to a particular caste are also largely unknown.

Haydak (1943) demonstrated that the quantity of food which the larvae receive is important to the differentiation of queens and workers. Weaver (1955, 1957, 1957b) has shown that the quality of the larval food initiates or controls differentiation, and he has proposed that both large amounts of food and food of a special quality are required for the differentiation of queens. Gontarski (1948), on the basis of microchemical studies of larval foods, concluded that the diameter of the cell determines the type of food the nurse bees place in it. Nold (1949) transferred larvae to cells of different sizes and concluded that the length of the cell is important in relation to the size of the larva. Weaver (1957) showed that the nurse bees recognize the caste of larvae. The literature on dimorphism and its causes has been reviewed by Ribbands (1953) and Johansson (1955).

MATERIALS AND METHODS.—A commercial strain of Italian honey bees (*Apis mellifera* L.) was used in a study of the effects of several factors on the differentiation of queens. The techniques employed in rearing the queens were the same as those described by Weaver (1957b), except that the larvae were single-grafted unless otherwise stated. When the queens emerged, they were placed directly in a deep-freeze and kept there until they could be dissected. The method of dissecting the queens and measuring the extent of dimorphic development were the same as those described previously (Weaver 1957b), except that the queens were not placed in a fixative, and only the distal ends of the ovaries were cut off before the ovarioles were separated and counted.

Worker larvae were placed in glass cells 6½ mm. in diameter and 9 mm. deep, and given to a queenless, broodless colony. Fifteen hours later a wax extension the bees had built on the mouth of each cell was cut off. This was repeated every 2 hours for the next 32 hours, and the cells were left in the queenless colony until they were sealed. Glass cells 6½ mm. in diameter at the mouth, 9 mm. in diameter, at the middle, and 24 mm. deep, and glass cells 10 mm. in diameter and 24 mm. deep were grafted with worker larvae and left in queenless colonies until they were sealed. Only cells of one size were reared in each queenless colony, and cells without larvae at the end of the first and second day were regrafted. The control queen cells were left in the queenless colony for only 1 day, and then they were transferred to a finishing colony. The queenless colonies and finishing colony were given cakes made of pollen and water (Weaver 1956).

The more-liquid royal jelly around and immediately under the larvae was pipetted from queen cells for use in other experiments. Queens were reared in cells from which the surface royal jelly was removed on the second day, on the first and third day, and on the second and fourth day after they were grafted. Other cells were grafted with larvae less than 12 hours old, and on the following day all of the royal jelly was removed from the cells. The larvae were then returned to the cells, placed in finishing colonies, and all of the royal jelly was removed once each day until the cells were sealed. The adults that emerged from these cells were dissected and measured.

Larvae were grafted into three frames of queen cells in a queenless colony, left there for a day, and each frame was moved to a separate finishing colony. Two of the frames were then double-grafted (Weaver 1957b), and queens were reared from the cells on the three frames.

RESULTS AND DISCUSSION.—Queens were reared in glass cells by queenless colonies of bees late in June, 1953. Bees in finishing colonies removed larvae from glass cells,

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and even the queenless colonies reared only a small percentage of the larvae to maturity. The bees built wax extensions on the mouths of the $6\frac{1}{2}\times9$-mm. cells. When these were cut off, the bees would rebuild them completely within 2 hours, but when royal jelly and larvae virtually filled the cells, the bees would remove the larvae if the wax extensions were cut off.

The bees built wax collars about 3 mm. wide and 1 mm. thick just inside the mouth of the 10×24-mm. glass cells. If a collar was removed, it was immediately rebuilt. The collar may have helped the bees cling to the sides of the cell when feeding the larvae. The bees would not seal these cells, and when a larva descended from the food to spin a cocoon, it would fall from the mouth of the cell if a cap was not affixed to it. In these cells, and the $6\frac{1}{2}\times24$-mm. glass cells, some of the pupae were wet by the royal jelly, and some of them faced toward the bases of the cells.

The means and their standard errors, and the F values for the differences between treatment means of some of the measurements of these queens are presented in table 1. It will be noted that the bees reared in glass cells were slightly more worker-like in some measurements than their controls. In addition to the characteristics shown in the table, these queens differed significantly in basitarsal index, adbomen-breadth, and diameter of spermatheca. They did not differ significantly in tongue-length or in the number of barbs on the sting.

In another experiment conducted late in July, 1953, adults were reared from larvae that were 3 days old when they were grafted into queen cells. These individuals were similar to those reared from larvae of the same age and reported by Weaver (1957b). Queens reared at the same time in cells from which the surface royal jelly had been pipetted once or twice differed little from their controls. No data on any of these queens are given in table 1.

Late in August, 1953, the surface royal jelly was pipetted from queen cells on the first, and again on the third day after they were grafted, and adults were reared from the larvae in the cells. They differed only slightly from the controls. Other adults were reared in cells from which all of the royal jelly was removed daily. It will be noted in table 1 that these individuals were much more worker-like in their characteristics than their controls. In addition to the characteristics shown in the table, these bees differed significantly from the controls in basitarsal index and abdomen-breadth, but not in spermatheca. diameter, tongue-length, or number of barbs on the sting. Some of these bees had mandibles with only slight indentations, and some were worker-like in the chaetotaxy of the metathoracic legs.

The cells yielded a mean of 35 mg. of royal jelly 1 day after they were grafted, 72 mg. the second day, 121 mg. the third day, 170 mg. the fourth, and 138 mg. on the fifth day after being grafted. Seventy-three per cent of the larvae from which jelly was removed on the first day were being fed by the bees on the second; 92% of these survived till the third day; 70% of these till the fourth, and 18% of these till the fifth day.

Early in March, 1954, an experiment was conducted to test the value of double grafting (Weaver 1957). The colonies available at the time were rapidly expanding brood-rearing, but it was more than a month before they reached their peak populations. It is probable that the bees were feeding about the maximum number of worker larvae that they were able to care for. One frame of the double grafted larvae was placed in the most populous finishing colony available, and one in the least populous; the colony that received the single grafted cells was intermediate in strength. The queens reared from double grafted larvae in the strong finishing colony had significantly larger spermathecae than the other queens, but there was no other significant difference in the queens. All of these individuals were rather poor queens anatomically, as shown by the measurements given in table 1.

In comparison with a putative anatomically ideal queen, the normal queens produced in June were better queens anatomically than those produced in July, the latter were better than those produced in August, which in turn were better than the queens produced early in March. Since there were many changes in the colonies as the summer progressed, and from summer to early spring, it would be difficult to assess the importance of the individual factors responsible for these variations. Visual observations indicated that when there was little pollen being brought into the hive, the bees in a queenless colony placed more royal jelly in queen cells if the colony was given a pollen cake than if it was given combs well filled with stored pollen. The number of bees and amount of brood in the colonies, the physiological condition of the adults, and the amount of nectar being brought into the hive are probably partially responsible for the type of queens that a colony rears.

Although the intermediates between normal queens and normal workers that have been produced by various treatments differ widely among themselves, there is no evidence that the type of variation depends upon the treatment the larvae receive (*e.g.*, these data and those presented by Weaver 1957, 1957b). This probably results

Table 1.—Means and their standard errors of some measurements of queen honey bees, with F values for the differences between treatment means.

Treatment	No. Bees	Abdomen Length (mm.)	Diameter Right Ovary (mm.)	No. Ovarioles Both Ovaries	Mandible Gland Length (mm.)
Glass cells $6\frac{1}{2}\times9$ mm.	9	7.81±.19	1.78±.11	227±26	1.82±.06
Glass cells $6\frac{1}{2}\times24$ mm.	5	7.40±.84	1.60±.22	229±35	1.59±.13
Glass cells 10×24 mm.	3	7.70±.55	1.39±.14	201± 3	1.71±.06
Controls	12	8.20±.18	2.01±.10	376±16	1.74±.03
F values		<1	4.25*	7.79**	1.93
Surface jelly removed twice	5	7.66±.24	1.67±.10	268±32	1.73±.06
Jelly removed daily	15	6.45±.15	1.34±.11	225±13	1.39±.04
Controls	9	7.82±.32	1.71±.08	307±11	1.66±.07
F values		13.18**	3.50*	7.99**	9.62**
Single graft	16	8.63±.12	1.88±.07	284±16	2.03±.07
Double graft strong colony	15	8.78±.14	1.77±.08	289±14	1.88±.05
Double graft weak colony	11	8.65±.10	1.93±.07	279±10	1.90±.08
F values		<1	1.38	<1	1.52

* Significant at the .05 level.
** Significant at the .01 level.

from the nature of the bees' developmental physiology, though all treatments, despite apparent differences, could affect the developmental physiology in the same manner. The larvae in glass cells may have received food of a slightly different quality from that given to normal queen larvae, but the larvae in the long glass queen cells had less royal jelly in their cells throughout development than larvae in normal cells. Furthermore, it is doubtful if the larvae in any of the glass cells could reach enough of the royal jelly to feed normally after they had spun cocoons. It seems probable that the queens reared in cells from which the royal jelly was removed daily were poor queens anatomically because of the small quantities of food that they received, but the quality of the food could have been altered by the nurse bees under the stress of producing maximum quantities of royal jelly, or because they consumed less food, the larvae may have received less of a particular substance necessary for the differentiation of normal queens.

SUMMARY.—Queen honey bees reared by queenless colonies of bees in glass cells of three different dimensions were more worker-like in several anatomical characteristics than their controls. Queens reared in cells from which the more-liquid surface royal jelly was pipetted once or twice during larval development differed little from their controls, but queens reared in cells from which all of the royal jelly was removed once daily were much more worker-like than their controls. Queens reared by the double-grafting technique were very similar to those reared by single-grafting. Queens reared as controls under different conditions differed from each other in some characteristics. The factors that may have caused the differences in the queen are discussed briefly.

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The Relative Susceptibility of the Life History Stages of the Rice Weevil[1] to Certain Fumigants[2]

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Although fumigants have long been used to rid grain of insect infestations, surprisingly little is known about the reaction of the developmental stages of most stored grain insects to the common fumigants. Cotton (1932), Lindgren & Shepard (1932), Lindgren (1935), and Sun (1947) reported studies comparing the relative susceptibility of the various stages of the confused flour beetle, *Tribolium confusum* Duv., to fumigants. Fryer (1947) and Brown (1952) indicated that for any one of the dozens of economic species requiring fumigation, the life history stages may differ in their resistance to fumigants, and that the variabilities among these stages of any one species may be as great as those among the same stage of different species. This paper reports the results of an investigation to determine the relative susceptibility of all stages of the rice weevil to two fumigants.

MATERIALS AND METHODS.—The test insects were reared from parental stock produced by mixing adult rice weevils taken from cultures maintained by the Kansas Agricultural Experiment Station and the U. S. Department of Agriculture Stored Products Insect Section, Manhattan, Kansas. Cultures were maintained at 82° F. (±2°) in 1-quart, wide-mouth Mason jars containing approximately ½ inch of Pawnee wheat. Two hundred active adults between 20 and 30 days old were placed in each jar to oviposit for 3 days before being removed. The age of the test insects could be determined within 1 day. New cultures were made every 3 days.

The wheat for future tests was held at approximately 0° F. for several days to kill incipient insect infestations. After the moisture content of the wheat was adjusted to 13.5%, it was stored in the rearing room where the fumigations were conducted.

Cultures for fumigation were selected by dissecting kernels from cultures of known age until one was found containing the instar desired. One hundred additional infested kernels from this culture were dissected to determine the percentage of individuals present in the same developmental stage. Only those cultures containing at least 75% of the immature forms in the desired stage were used for fumigation. During this study it became apparent that as the age of the culture increased, the

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greater was the likelihood of having more than one stage or instar present. For example, it was relatively easy to obtain cultures having all the individuals in the egg stage, but a culture having all the individuals in the pupal stage was never found. This condition should be taken into account when considering the data reported here.

Since each larval instar was treated separately, it was essential that the presence of these stages be accurately determined. Under the conditions maintained during this investigation, development from egg to adult required an average of 28 to 30 days. The critera used to determine the larval instars were age of the culture, head capsule measurements, and the amount of damage found in the infested kernels. Before any fumigations were attempted, numerous daily dissections of infested kernels from cultures of various ages were made and the larvae examined in order to become familiar with their distinguishing characteristics. Particular emphasis was given to their relative size, appearance, location in the kernels, and feeding damage.

When a certain immature stage living within the kernels was needed for fumigation, samples of grain were taken from several cultures, dissected, and the stages present and their relative numbers determined. After a specific culture was found having the insects present in the desired developmental stage, the grain in the individual jars of this culture was mixed thoroughly for uniformity before the samples were drawn for fumigation. It was found during preliminary trials that four replications each with 250 kernels provided enough insects to make a statistically valid test.

The kernels selected for fumigation were placed in small cages of 60-mesh screen wire sealed with heavily waxed stoppers. The adults were also fumigated in this type cage. The cages containing the immature or adult weevils were buried prior to fumigation for at least 6 hours in bulk grain stored in the rearing room to condition the infested kernels and the insects to the humidity and temperature maintained there.

Fifteen- to twenty-day-old adult weevils were chosen for fumigation. Only those weevils that were able to climb the sides of an enameled pan were selected. Two series of tests were made with each fumigant, one with the adults in the absence of grain, and one in the presence of a small quantity of grain. Where the adults were fumigated in grain, they were placed into the fumigation cages along with 7.30 grams of wheat, which is the average weight of 250 kernels. Twenty-five adults were used in each of four replicates.

All fumigations were conducted in clean 1-gallon jars. The jars were stored in the rearing room for at least 6 hours prior to fumigation to allow their atmosphere to reach temperature equilibrium with that of the rearing room. This equilibrium was maintained in the jars during the time required for the introduction of the fumigant. Just prior to fumigation, the caged insects were removed from the bulk grain in the rearing room and were placed in the fumigation jars along with a small uniform wad of cotton. Only one cage was placed in each jar. The desired amount of fumigant was added to each jar by extending a pipette to the bottom of the jar and releasing the fumigant on the cotton. As soon as the pipette was emptied, it was withdrawn and the jar quickly and tightly sealed to minimize the loss of the fumigant vapors. Since a jar could be fumigated and sealed in approximately 30 seconds, very little fumigant was lost.

The jars used as fumigation chambers were sealed by standard two-piece rings and rubberized lids. The volume of the jars ranged from 3.91 liters to 3.93 liters, with a mean volume of 3.915 liters.

The fumigants were introduced into the jars in the laboratory. As soon as the jars were sealed, they were immediately transported to the insect rearing room for storage until the 24-hour fumigation was completed.

As the gallon jars were being opened at the end of the fumigation period, the fumigant odors could be strongly detected. It was, therefore, assumed that the vapors diffused so that the concentration was uniform in the jars; however, no tests were made to confirm this assumption.

After the fumigation period was completed, the insects were transferred from the screen cages to 1-ounce tin salve boxes which had two small holes for aeration. In tests with adults these boxes were furnished with a small quantity of untreated grain. After the boxes were sealed and labelled, they were buried in bulk grain taken from the regular culture media and stored in the rearing room for 7 days. In cases involving adults, mortality resulting from fumigation was determined before they were placed into the salve boxes. A second mortality count was made after 7 days. The immature forms were held in these boxes until emergence was complete. The determination of the lethal action of the fumigants upon the immature forms of the rice weevil was made by comparing the number of adult weevils emerging from the treated samples with the number emerging from the untreated samples.

Adult mortality was determined by the method reported by Sun (1947). Only those insects showing no signs of movement were considered dead. Adjustments for mortality occurring in the check cages were made according to Abbott's formula (Abbott 1925).

The two fumigants used were ethylene dichloride (CH_2ClCH_2Cl) and carbon tetrachloride (CCl_4). Both chemicals were of the grade commonly used as analytical reagents.

Prior to each treatment the fumigants and pipettes to be used were chilled to approximately 0° F. to minimize volatilization at time of application. The fumigants were dried with calcium chloride and distilled before each application to remove any moisture resulting from condensation.

All the tests were designed to determine the LC_{50} (median lethal concentration) of each fumigant for each stage. The data were analyzed and the LC_{50} values determined by the methods of probit analysis as reported by Bliss (1935).

RESULTS AND DISCUSSION.—The relative susceptibility of each stage to the two fumigants is reported in table 1. The LC_{50} values indicate the order of resistance from greatest to least to carbon tetrachloride to be pupae = egg = fourth instar larvae > third instar > pre-emergence adults > first instar = second instar > adults. Where differences are shown in resistance, they are significant at the 5% level. The slopes of the lines (b values) suggest that the first and second instars were most sensitive to change in dosages while the eggs, third instar larvae, pupae, pre-emergence adults, and adults were least sensitive.

Table 1.—Median lethal concentration, confidence limits, and slopes (b values) of carbon tetrachloride and ethylene dichloride for the various developmental stages of the rice weevil.

STAGE	SLOPE (b)	95% CONFIDENCE LIMITS MG./LITER		
		Lower	LD_{50}	Upper
Carbon tetrachloride				
Egg	4.85	86	92	96
1st instar larva	13.56	48	51	54
2nd instar larva	11.91	44	46	49
3rd instar larva	3.45	70	77	81
4th instar larva	7.89	93	96	99
Pupa	2.93	95	103	111
Pre-emergence adults	3.33	60	65	69
Adults out of grain mass	3.37	30	33	38
Adults in grain mass	3.43	31	33	36
Ethylene dichloride				
Egg	6.91	17	19	20
1st instar larva	6.44	16	18	21
2nd instar larva	5.65	15	16	18
3rd instar larva	7.52	26	28	30
4th instar larva	2.55	11	13	14
Pupa	3.08	12	13	13
Pre-emergence adults	1.57	8	12	15
Adults out of grain mass	2.08	12	13	16
Adults in grain mass	1.77	12	13	16

The order of resistance of the various stages to ethylene dichloride was found to be third instar larvae > egg = first instar = second instar > adults = pupae = fourth instar = pre-emergence adults. The b values indicate that eggs, first, second, and third instars are more sensitive to change in dosages than are the other stages.

The comparative toxicity of the two fumigants to the various stages is indicated by the LC_{50} values reported in table 1. These data suggest that ethylene dichloride is much more toxic to all stages than carbon tetrachloride. It should be noted that the order of resistance of each stage to the fumigants was not the same. For example, the pupa was the most resistant stage to carbon tetrachloride, whereas, the third instar larva was the stage most resistant to ethylene dichloride. The respective LC_{50} values for each stage indicate an appreciably wider range in dosage levels for carbon tetrachloride than for ethylene dichloride. For example, the LC_{50} values for carbon tetrachloride ranged from 33 mg. per liter for adults to 103 mg. per liter for pupae. The range for ethylene dichloride was much narrower, being 13 mg. per liter for adults and 28 mg. per liter for third instar larvae.

The differences in degrees of resistance may be better illustrated by a comparison of the quantities of fumigant required to obtain LC_{50} values for some of the stages. The data indicate that approximately twice as much carbon tetrachloride was required to obtain the LC_{50} for pre-emergence adults as for the adults. Dosages approximately three times that reported for the adults were required for pupae, eggs, and larvae in the fourth instar. The range in dosage of ethylene dichloride required to obtain LC_{50} values for each stage was not so wide as that of carbon tetrachloride. The most resistant stage (third instar) required a dosage approximately twice that required by the most susceptible stage (adults). Except for the third instar larvae, differences in resistance of the stages to ethylene dichloride appeared to be of little practical importance.

Several workers have reported data which suggest that the order of resistance to fumigants may be related to the respiratory rate of the stages being tested. Cotton (1932) indicated that the order of the respiratory rate of *Tribolium confusum* was paralleled by a similar order of susceptibility. He suggested that the stage with the highest metabolic rate was least resistant. Sun (1947) also indicated a close correlation between susceptibility of insects to fumigation and their rate of metabolism; however, Lindgren (1935) presented data indicating that this relation was not necessarily true and depends a great deal upon the fumigant being tested. Thus, it appears that many factors may influence the susceptibility of any one stage of a species to fumigation. The results of this investigation indicate that there were great differences in susceptibility among the various stages of the rice weevil to the fumigants tested, and that these differences were not of a similar nature when comparing the relative toxicity of the two fumigants to the same stage.

SUMMARY.—Methods are described for fumigating and determining the resulting mortality of the various larval instars, pupae, pre-emergence adults, and free adults of the rice weevil, *Sitophilus oryza* (L.) to carbon tetrachloride and ethylene dichloride. The tests were designed to obtain the LC_{50} values for each stage and instar. The method of probit analysis was used to obtain these values.

The LC_{50} values for carbon tetrachloride indicated that the descending order of resistance of the various stages to this fumigant was pupae = eggs = fourth instar larvae > third instar > pre-emergence adults > first instar = second instar > adults. The slopes of the lines suggested that the first and second larval instars were more sensitive to changes in dosages than the other stages.

The LC_{50} values for ethylene dichloride indicated that the descending order of resistance to this fumigant was third instar larvae > egg = first instar = second instar > adults = pupae = fourth instar = pre-emergence adults. The slopes of the various curves suggested that eggs, then first, second, and third instars, in that order, were more sensitive to changes in dosages than the later stages.

Ethylene dichloride was considerably more toxic to all stages than carbon tetrachloride. Furthermore, each stage did not react in a like manner to the two fumigants, as illustrated by the third instar, which was most resistant to ethylene dichloride, while the pupa, fourth instar, and egg stages were the most resistant to carbon tetrachloride. The adults were among the most susceptible stages to both fumigants. The early instars appeared to be more sensitive to both fumigants than the later stages.

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Ecological Observations of the Valley Black Gnat, *Leptoconops torrens* Townsend[1]

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The valley black gnat, *Leptoconops torrens* Townsend (Certopogonidae = Heleidae), is a small biting insect which causes considerable annoyance to man and domestic animals in many areas of California.

The gnats are daytime feeders, and the females appear to readily attack any accessible warm blooded animal. All areas of the anatomy are bitten, including the ears, around the eyes, scalp, and the axillary sites. Clothing offers little protection as the gnats easily enter any convenient opening. They crawl over the skin, usually biting at the beltline, neckline, under the socks and at other spots where the clothing comes in close contact with the skin. The bite is not painful but it is followed shortly by an intense, persistent itching which may continue from a week to 10 days. An allergic factor associated with the bites invokes a variety of individual responses. Secondary infections from scratching, particularly in children, as well as the allergic manifestations, frequently require medical attention. During the peak of the gnat season, construction projects have been halted and agricultural workers have refused to work in areas of intense biting activity. Country clubs have reported financial losses due to reduced patronage of the golfing membership during the gnat season.

Although the limits of the gnat's geographic distribution in California have not been completely or accurately defined, the records of its occurrence indicate a discontinuous distribution in many areas, particularly along the west side of the Central Valley and in a number of the smaller coastal valleys of northern California. In the Central Valley it appears to be associated with the distribution of the fine textured clay soils in poorly drained low lying land. Such soil conditions are found commonly along the west side of the Central Valley approximately from Willows, Glenn County on the north to the area of Tulare Lake on the south. Outside of California this species has been recorded from Uvalde and Dallas, Texas; Las Vegas, Nevada; and New Mexico and Colorado. There are no known records outside of the United States. The adult flight period is seasonal, occurring in the spring and early summer. While seasonal variations in weather conditions influence the time of emergence and the duration and intensity of the adult flight period, the gnats seldom appear before late April or early May and usually disappear in July. June is usually the month of peak adult activity at Davis, California.

Only the adult stage of the gnat was known until 1944 when the first larvae were discovered in clay adobe soil at a depth of 20 inches near Davis, Yolo County, Calif., by Smith & Lowe (1948). An intensive study by Smith of this larval habitat revealed considerable information on the life history of *L. torrens*. Larvae were observed to inhabit clay-adobe soil to depths ranging from 15 to 30 inches. The larval period was found to extend at least 2 years with summers spent in immobile aestivation. The pupal stage was not discovered. The evidence indicates that the pupation period is very brief and occurs upon drying and cracking of the soil following the end of the rainy season (usually in May). The adult gnats emerge and crawl out from the soil cracks. Engorged, mated females re-enter the crack system and oviposit. It is presumed that with the advent of rains in the fall, the eggs hatch and the young larvae burrow through the mud in the crack faces. If environmental conditions act to delay soil cracking, gnat emergence may fail to materialize. In this event the larvae apparently enter diapause, a condition which may, depending on the circumstances, extend for several years. Attempts to rear the gnat in the laboratory have not been successful.

Because of the distress and discomfort occasioned by the persistent biting of the gnats, health departments, mosquito abatement districts and other public agencies located in gnat-infested territory receive many requests to control the pest. Attempts made to control the larvae and adults have included DDT dusting of fields suspected of being gnat sources, DDT thermal aerosoling, and mist spraying by airplane for adult control. As far as can be determined, the results of these operations were either unsuccessful or inconclusive.

The most important single deterrent to effective planning and organizing of control operations has been the difficulty of finding, and delineating with some degree of precision, the locations of the gnat sources. With this need in mind a survey aimed primarily at locating sources through recovery of larvae was undertaken in the area of Davis, Yolo County, in October 1955. At that time no gnat source other than the one discovered by Smith in 1944 had been found either through recovery of the larvae from the soil or by collection of the adult gnats in emergence traps appressed to the earth. During the gnat

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emergence season of 1956, the objective was broadened to include soil emergence trap studies and observations of adult occurrence.

MATERIALS AND METHODS.—The survey plan called for obtaining soil samples by pick and shovel and soil auger excavation in suspected gnat sources, and examining the samples for the presence of larvae and pupae. Several methods of examination were attempted. Visual examination of soil samples broken by hand into pea-size particles, although time consuming and tedious, yielded positive results. Two other methods tried were unsatisfactory. These consisted of attempts to extract the larvae by screening a soil slurry through a series of Tyler screens and a flotation method utilizing a zinc sulphate solution.

The method employed for determining adult emergence from the soil consisted of setting emergence traps in suspected gnat sources and recording the number of gnats collected each day. The traps were adapted from the type used by Smith (1933) in pear thrips studies. They are pyramidal shaped, 1 square yard at the base, of wood frame construction covered by waterproof sisal Kraft paper. The apex of the trap was fitted with a removable shell vial which collects the emerging adults. The glass at the base of the vial was reshaped into an inverted cone in the vial and provided with an opening at the tip of sufficient size to admit an adult gnat. The mouth of the vial was plugged with a cotton wad to permit the escape of moisture which condensed in large droplets in the vial when a cork stopper was used. In placing the traps the base was recessed into a groove cut about 2 inches deep into the soil surface. Then fine dry sand was poured around the outside of the trap base as a seal to prevent the emerging gnats from escaping laterally through the crack system.

RESULTS.—In October 1955, the site of the gnat source (Covell Ranch) discovered by Smith at Davis in 1943, was inspected. This was originally a shallow swale-like depression of clay-adobe soil located at the low end of a field planted to barley. As a result of land leveling in October 1952, incident to preparing the land for irrigation and planting alfalfa, the site was covered with topsoil. However, the original site was located and a hole dug 18 inches deep through the topsoil fill down to the former clay-adobe soil surface. From this level the excavation was continued an additional 18 inches, where three larvae were recovered. This finding was unexpected since it was felt that the irrigated 18-inch layer of topsoil covering the clay adobe would, in effect, act as a seal to prevent both the emergence of adults and reinfestation by ovipositing females. Since larvae were recovered at the same approximate depth from which they had been taken 10 years previously, it was concluded that either one of two possibilities accounted for their presence; first, that the larvae had entered a diapause and were the survivors of a generation of gnats which hatched from eggs oviposited before the leveling and irrigation of the land was completed 3 years before; second, that despite the physical change produced by land leveling and irrigation, there was sufficient cracking of the topsoil to permit limited gnat emergence and reinfestation to occur. Subsequent observations of gnat emergence from this site in 1956 (see table 2) gives credence to the first possibility and adds further evidence to support the diapause phenomenon first advanced by Smith.

A search for new gnat sources was conducted over a wide area in the vicinity of Davis using as a guide the knowledge of the soil conditions at the Covell Ranch source and information from local farmers concerning the locations of intensely infested areas. After many negative results, one larva was finally recovered on November 21, 1955, at a depth of approximately 36 inches, in a shallow swale-like depression on uncultivated land. Subsequently, larvae were found in similar situations at three other widely-separated locations within a 6-mile radius of Davis. Although the soil was examined from all parts of the swales from the upper edge down to the center, it was noted that the larvae were most prevalent in the soil near the marginal areas.

As shown in table 1, larvae were recovered at soil depths ranging between 18 and 36 inches. In three instances—the Glide (later abandoned because of damage to the traps by livestock), Buckley and Saxon station locations—the sites of recovery were in shallow, poorly drained, swale-like depressions located in uncultivated nearly flat terrain. These situations are technically referred to as slight swale areas lying in a basin topography. The soil in the swales is composed of basin clays derived from fine deposits which are sticky and tenacious when wet, becoming tough and hard and deeply fissured when dry. The outline of the swales is distinguishable from the surrounding higher land by the flora which, in the swales, is thinly distributed and botanically dissimilar during the spring and summer seasons. Tests of soil samples taken at the 2-foot-level revealed an alkali condition in all four breeding places. The pH reactions were alkaline in all cases. However, the salinity values varied widely from a low of 0.420 p.p.m. at the Buckley source to a high of 4200 p.p.m. at the Walsh Airfield.

The Walsh Airfield gnat source, although of the same basic soil character as the other sites, tested highest in alkali salts derived from sodium carbonate. The dominant plant growth was salt grass, *Distichlis spicata*, both in the swales and the surrounding higher land. The most common plants found in the other breeding places were species indigenous to wet clay soils and the dry beds of vernal pools. These are listed below:

Hayfield Tarweed, *Hemizonia congesta*
Lupine, *Lupinus* sp.
Gold Fields, *Baeria fremontii*
Sacramento Pogogyne, *Pogogyne ziziphorides*
Sunflower family, *Psilocarphus oreganus*
Tricolor Monkey Flower, *Mimulus tricolor*
Douglas Pogogyne, *Pogogyne, douglasii*

Table 1.—Recovery of *Leptoconops torrens* larvae from soil samples taken in the area of Davis, Calif., 1955–56.

LOCATION	DATES OF COLLECTION	SOIL DEPTHS	SOIL TYPE
Covell Ranch	October, 1955	36″ (18″ fill)	Clay adobe
Glide Ranch	November, 1955	18″ to 32″	Capay clay
Buckley Ranch	April, 1956	36″	Capay clay
Saxon Station	May, 1956	28″ to 32″	Capay clay
Walsh Airfield	May, 1956	21″	Dunnigan clay

Table 2.—Collections of *Leptoconops torrens* from soil emergence traps near Davis, Calif., 1956.

Month and Day	Locations					Total
	Saxon	Covell	Walsh	Buckley	U.S. Air Force	
May 29–31	4		8			12
June 1–3	3		7	1	1	12
June 4–6	11	1	4		1	17
June 7–9	56	2	0	1		59
June 10–12	18		1		9	28
June 13–15	8		0			8
June 17–18[a]	6		1			7
Total	106	3	21	2	11	143
No. of Traps	7	4	5	4	5	25

[a] No collections made on June 16; no gnats recovered after June 18.

Recovery of Gnats from Emergence Traps.—On May 28 emergence traps were placed at all sites positive for larvae and in one additional area which appeared to be an extensive gnat source.

As shown in table 2 gnats were captured from all of the locations trapped. In two of the five places, emerging adults were collected on May 29, the first day that the traps were placed. Thereafter collections were taken almost daily until June 18, a period of 21 days. Although the traps were kept in operation until June 29, results were negative after June 18. This compares with the emergence period of 17 days from May 21 to June 10, observed by Smith in 1943. The relatively late 1956 emergence date may have been caused by the heavy rainfall and floods experienced in northern California during the winter of 1955–56, which saturated the soils and delayed cracking.

The findings at the Covell Ranch, where six gnats were recovered from three traps, is significant since it suggests that despite land leveling and irrigation of gnat sources some emergence may still occur. However, the few gnats captured, when compared with 213 gnats taken in the same area by Smith in 1945 from three traps, suggests that land leveling and irrigation may be effective methods for controlling gnats at the source. This conclusion is further supported by the fact that only a few gnats were noted in flight during the 1956 emergence season at the Covell Ranch. Prior to the land leveling operations in 1952, this area had always experienced extremely high adult densities and was regarded as one of the most heavily infested gnat areas in the vicinity of Davis.

The gnat emergence from the Saxon station was sufficiently high and sustained throughout the season to suggest a pattern of emergence and to justify closer examination of the results.

The gnat source at Saxon station is a slight swale area within a basin topography. In the winter of 1956 the area was flooded by overflow from the Sacramento River. The soil is dominantly saline (chlorides and sulphates) and is characterized by poorly developed drainage. It is located in a transition zone between Capay clay and Clear Lake adobe soil types. These clays are described by Cosby (1931).

On May 28, four emergence traps were placed in a row about 50 feet apart, from the center of the swale to a point about 25 feet beyond the outer margin. The outline of the swale was roughly circular and measured about 350 feet in diameter. The trap placed about 25 feet beyond the perimeter was used as a control. The number of gnats recovered from each of the traps is presented in table 3.

The outstanding feature of the Saxon collection data is that trap No. 3 captured nearly 84% of the total of all the gnats collected from the four traps. This trap was situated about 25 feet from the edge of the swale-like depression. The remaining three traps accounted for only 16% of the gnats collected during the 21-day emergence period. Smith obtained similar results at the Covell Ranch in 1943. He found that out of three traps, placed in a row 50 feet apart in a clay-adobe swale-like land depression, the trap located near the edge collected 209 from a total of 213 gnats.

Since trap No. 4, located 25 feet beyond the edge of the swale area as a control, had captured one gnat on June 5, three additional traps were set even further beyond the margin on the highest elevation of the land grade in the field. This was done on June 6, nine days after the trapping was begun, at the time of peak emergence. Two gnats were collected from these traps, one each on the first and second day after placement but none thereafter. The traps in the swale area, however, continued to collect gnats for a period of 10 days. These results suggest the possibility that the gnats collected on high ground had emerged elsewhere and had found harbor in the soil cracks over which the traps were placed.

Pupal Stage.—Despite many hours of careful searching for the pupae, none were recovered from the soil samples, and attempts made to induce larvae to pupate were unsuccessful.

Adult Gnat Flight Activity.—A few gnats were first noted on May 26. Flight activity was sporadic and remained light until June 1 when a sharp increase in the population was observed. From the standpoint of the general public, which began to take notice and complain of gnat bites in the area of Davis, this marked the beginning of the gnat season for 1956. Except for fluctuations in flight activity caused by wind and temperature conditions, there was no noticeable diminishing of the population until the end of June, after which it declined rapidly. Although a few gnats were observed as late as July 23 at the Saxon station, there were no signs or reports of their presence elsewhere in the Davis area. There was no flight activity observed at night. The New Jersey light trap, which was operated at the Walsh Airfield source during the gnat season, did not collect gnats. It was noted that the gnats rarely entered buildings to bite.

Adult flights and biting activity were most pronounced when high temperatures (85° F. and over) prevailed usually during the afternoon. No flight or biting was observed when the temperatures dropped below 70° F.

On windy days flight and biting activity was greatly reduced regardless of temperature conditions.

Table 3.—Adult gnat collections from individual emergence traps placed at Saxon Station.

Trap No.	Number of Gnats Recovered			Per Cent of Total Recovery	Approximate Position of Traps from Center of Swale
	♀	♂	Total		
1	1	0	1	0.9	50 feet
2	2	11	13	12.6	100 feet
3	32	54	86	83.6	150 feet (25 feet in from edge of swale
4	2	1	3	2.9	25 feet beyond edge of swale
Total	37	66	103	100.0	

DISCUSSION.—The information obtained in this survey adds further evidence in support of the belief that sources of the valley black gnat are associated with fine textured clay soils in low lying land with poorly developed drainage. It appears that the immature stages are not uniformly distributed throughout these soils but that they are restricted to swale-like depressions containing some alkali in which the soil fissures deeply on drying. However, other types of soil conditions and areas cannot be ruled out as possible sources until more extensive studies are made.

Adult emergence is greatly reduced and possibly prevented by land leveling, irrigation, and other cultural practices which alter the normal cracking of the soil in gnat breeding areas. However, under such conditions the mature larvae are apparently able to survive for at least several years by entering diapause.

The application of residual type insecticides to the gnat sources may prove effective as a control measure. Considerable experimental work needs to be undertaken, however, before any large-scale control operations can be recommended. Any attempt at control should also be preceded by a thorough survey to locate the specific sites of gnat sources. This is a difficult problem with the present known methods of identifying sources. The need for developing more efficient methods of extracting the larvae from soil samples is particularly urgent and should offer a productive field for laboratory research.

Improved methods of collecting the gnats should be devised to facilitate obtaining information on their flight range, seasonal occurrence and geographic distribution. The collecting methods in current use, such as sweeping with an insect net and collecting the gnats as they land on the body, are not satisfactory. Some type of trap, possibly employing a chemical attractant needs to be developed.

The discovery of the pupal stage and the successful rearing of the gnats in the laboratory is necessary to complete the knowledge of the life history and to obtain more accurate information on the specific environmental requirements of the immature stages.

SUMMARY.—A survey made to locate breeding places of the valley black gnat, *Leptoconops torrens* Townsend, in the area of Davis, Yolo County, California, resulted in the discovery of four new sources in 1955–56. Previously only one larval source had been discovered, also near Davis in 1944. Larvae were found in fine textured clay soils containing from light to heavy concentrations of alkali at depths ranging between 18 to 36 inches. The specific sites were in swale-like depressions in low lying terrain with poorly developed drainage.

Land leveling, irrigation, and other cultural practices which alter the cracking of the soil in gnat sources apparently prevents the emergence of adults. However, under such conditions the mature larvae apparently are able to survive by entering diapause. There was evidence found indicating that diapause may extend for at least 3 years.

Attempts made to induce larvae to pupate in the laboratory were unsuccessful, and no pupae were found under natural conditions.

Soil emergence trap studies showed an adult emergence period of 21 days from May 29 to June 18, 1956.

The adults were most numerous and active during the hottest periods of the daytime on still days. They bite outdoors and rarely enter buildings. They were inactive at temperatures below 70 F., at night, and during periods of high wind. The New Jersey type light trap was found to be ineffective as a collecting device.

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Observations on the Green Muscardine Fungus in Populations of *Oryctes rhinoceros* L.

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The green muscardine fungus, *Metarrhizium anisopliae* (Metch.) Sorokin, was first recorded by Metchnikoff in 1879, as parasitic on the wheat cockchafer, *Anisoplia austriaca* Hbst. Since Metchnikoff's early observations a large number of additional insect species have been reported as hosts of this fungus.

The first record of the attack of *M. anisopliae* on *Oryctes rhinoceros* L., was made by Friederichs in 1913 in Samoa. Considerable variation in the efficacy of this fungus as an agent for the biological control of *O. rhinoceros* has been reported; Friederichs (1913), Lester Smith (1936) and Simmonds (1941) advocated its use for the control of *O. rhinoceros*, whereas Bryce (1915, 1923), Johnpulle (1938) and Simmon (1939) considered it of little economic importance. This variation in virulence of the fungus was attributed to strain differences (Hopkins 1927). Recently Nirula *et al.* (1955) suggested that epizootics of this disease can probably be initiated, and the population of *O. rhinoceros*, reduced by the artificial dissemination of *M. anisopliae*.

CHARACTERISTICS OF THE FUNGUS.—*Metarrhizium anisopliae* belongs to the family Moniliaceae, and its systematic position is very close to the genus *Penicillium*. A variety *Americana* (Pettit 1895) and two forms, form a *major* and *minor* occurring on different hosts (Johnson 1915) have been recorded. Friederichs (1920) recognized two forms of *M. anisopliae* the long-spored and the short-spored ones. Most of the variations that have been noticed

are concerned with differences in spore sizes and in coloration.

The cultural characteristics of a few isolates of this fungus were studied by Radha *et al.* (1956). Considerable variation in the spore dimensions were observed among the isolates and they could be differentiated as the long-spored (10.6–12.0μ) and short-spored (3.5–8.2μ). In pathogenicity tests carried out by these authors only isolates from *O. rhinoceros* were capable of infecting the *Oryctes* grubs, while the cultures from other hosts were incapable of infecting these grubs. The possibility of the long-spored form, which exhibited specificity in pathogenicity, representing a physiological group was indicated, and Radha *et al.* suggested the institution of a var. *rhinoceros* for this group.

M. anisopliae grows readily on artificial media, but the rate of growth is poor. Radha *et al.* recorded the radial spread after 14 days of incubation in cultures grown on 2% potato dextrose agar, rice agar and groundnut shell agar at laboratory temperatures of 26 to 28° C. The spores are at first white, but gradually turn olive green as they mature.

TRANSMISSION AND SYMPTOMS OF INFECTION.—The transmission of the green muscardine disease in *O. rhinoceros* probably occurs by penetration of the integument. Radha *et al.* observed the mature green spores of the fungus in the intestines of the infected *Oryctes* larvae, which probably they swallowed while feeding. These spores did not show any signs of germination. Glaser (1926) reporting his studies of the green muscardine disease in silkworms and Wallengren & Johansson (1929) describing the manner in which *M. anisopliae* infects *Pyrausta nubilalis* (Hbn.) also stated that these insects acquired the infection through the integument.

The fungus infects the larvae, pupae and the adults of *O. rhinoceros*. The eggs are not attacked. The first discernible symptoms of infection in *Oryctes* larvae start appearing from 3 to 7 days after infection, and these consist of a change in color and appearance of dark brown patches on the cuticle. The normal color of the larvae is greyish white, and it changes to dull creamy white in the infected larvae. The patches may appear on any part of the body, and these usually spread over the other parts. Sometimes the patches may be quite small or altogether undeterminable. Marked nervous disturbances become evident in the later stage of the disease. Two or three days before death, the larva loses its appetite, becomes apathetic, makes no spontaneous movements and its irritability decreases more and more. A few hours before death, the larva wriggles out of its breeding material and lies helpless making irregular and feeble movements. Death takes place within 1 to 3 weeks after infection. The cadaver is soft and pliable, and if a portion of the integument is indented, it remains sunken, with little or no reaction. The body of the insect gets shrunken, wrinkled and finally becomes mummified. During the post mortem period a rapid growth of the fungus takes place filling the interior of the body. Hyphae force their way through the body wall and appear as a white covering on the surface of the integument 3 days after death. Finally the characteristic green conidia are formed and the entire body of the insect appears dark green (Nirula *et al.* 1955).

The pupae and adults of *O. rhinoceros* do not show well marked premortem symptoms. The post mortem symptoms, according to Nirula *et al.* (1955), consist of deformation of appendages and shrinkage of the body followed by the formation of the fungal fructification in *Oryctes* pupae. In adults the legs stiffen, the abdomen becomes rigid and a white fungal growth appears from the intersegmental membranes, from the joint of legs, around the neck and at the base of the antenna. The characteristic green conidia appear about a week after death.

EPIZOOTICS OF THE DISEASE.—Nirula *et al.* (1955) have reported that the green muscardine disease is prevalent on the West Coast of India and during the periods of S.W. Monsoon, it takes a fair toll of *Oryctes* larvae, pupae and adults.

Observations on the natural incidence of infection by this disease were recorded during 1956 and are presented in table 1, along with relevant meteorological data.

Table 1.—Natural incidence of infection of *Oryctes rhinoceros* by the green muscardine fungus in 1956, with relevant meteorological records.

Month	Per Cent Infection			Character of Sky			Humidity Per Cent		Temperature ° C.		
				Number of Days		Average Hours of Bright Sunshine per Day					
	Larvae	Pupae	Adults	Cloudy	Clear		Maximum	Minimum	Maximum	Minimum	Rainfall (Inches)
January	0	—	—	6	25	9.5	87	48	32	21	0.2
February	0.9	—	—	2	27	10.1	83	50	33	22	0.0
March	1.2	—	—	2	29	[a]	86	56	34	24	0.6
April	3.2	4	—	12	18	[a]	86	61	33	24	4.6
May	5	—	—	18	13	6.3	90	72	31	24	10.9
June	5.8	8	10	25	5	2.4	95	81	29	23	24.0
July	11.5	—	10	26	5	5.6	94	71	29	23	7.2
August	11	—	8	17	14	5.8	93	72	29	23	5.0
September	7	4	5	23	7	5.6	92	71	30	23	5.0
October	4.5	5	—	20	11	5.4	90	74	30	23	18.3
November	3	—	—	12	18	7.3	90	61	32	23	3.2
December	0.3	—	—	—	31	9.8	83	47	33	21	0

[a] Data not available.

There are a number of ecological factors which are believed to influence the spread of fungal infections, but of these temperature, humidity and rainfall are the most important ones. Steinhaus (1949) has stated that the growth and the fructification of this fungus are retarded by the rays of sun, which indicated that clouds also played a part in the spread of the disease. Hence in addition to temperature, humidity and rainfall, the conditions of the sky and average number of hours of bright sunshine were also recorded.

From the data given in table 1, it is evident that the highest natural infection in *Oryctes* larvae, pupae and adults was recorded from May to October. During this period the humidity ranged from 71 to 95%, temperature varied from 23 to 31° C. and the rainfall ranged from 5 to 24 inches per month. During this period the number of cloudy days was more than 50% and average number of hours of bright sunshine was about 6 in a day of about 10 hours duration. Another interesting observation recorded was that the maximum infection occurred when the sky remained overcast continuously for a number of days with intermittent showers of rain.

From these observations it appears that probably a relative humidity higher than 70%, a temperature range falling between 23 and 31° C. and cloudy weather with frequent rains may be collectively or individually responsible for the rapid spread of the disease. In the absence of this type of environment, the infection is either very low or almost absent, as was recorded during January and December 1956 (table 1). During this period the minimum relative humidity was generally below 50% and the temperature went beyond 32° C. During January the sky was overcast for 6 days with a rainfall of 0.2 inch and in December there was no rain and the maximum number of hours of bright sunshine was recorded during this month. The infection of 0.3% recorded on *Oryctes* larvae during December might have actually taken place sometime in November, when the conditions were slightly favorable.

USE AS A CONTROL AGENT.—Since the parasitic nature of *M. anisopliae* was demonstrated various workers have tried to use it for the control of a number of crop pests. Krassilstschik (1886, 1888) used this fungus for the biological control of the sugar beet curculio and reported a mortality of 50 to 80% in the field. Further attempts to use *M. anisopliae* against various insect pests according to Steinhaus (1949) were made during the early years of the 20th century in Java, Hawaii, Trinidad and Puerto Rico. In some of these cases however the fungus did not prove beneficial as a practical means of control.

Attempts to control *Oryctes rhinoceros* by means of this fungus were made in Samoa, Ceylon and India, with varydegrees of success (Friederichs 1913, 1920, Bryce 1915, Lester Smith 1936, Johnpulle 1939, Nirula 1955). The application procedure consisted in placing infected beetles and grubs in trap piles of rotting coconut logs, decaying vegetable matter, and cattle droppings placed at random throughout the coconut estates. The beetles gathered in these traps for egg laying, and developing larvae were killed by the fungus.

Since the fungus is widely distributed in nature, and its spores, according to Steinhaus (1949), remain virile for a period of more than 3 years, many workers felt that further attempts to distribute the fungus artificially would not yield any significant result. The investigations reported herein indicate that the incidence of the green muscardine disease is not only dependent on the occurrence of the particular strain of the fungus pathogenic on *Oryctes* but that it is also correlated with a variety of environmental conditions; such as an atmosphere saturated with moisture, a temperature of about 27° C., and an overcast sky. In view of these observations the earlier conception of Nirula *et al.* of initiating an epizootic of this disease may prove successful by the dissemination of the var. *rhinoceros* of this fungus.

The use of micro-organisms in the control of insect pests, besides being inexpensive, has been almost entirely harmless to animals and plants in the past but each untried pathogen should be thoroughly tested for this possibility. The potential danger of some chemical residues to the host plants or to the consumers of plants is not a factor in microbial control.

SUMMARY.—The possibility of utilizing the green muscardine fungus *Metarrhizium anisopliae* (Metch.) Sor., in the biological control of *Oryctes rhinoceros* L. has been suggested in this paper.

The natural incidence of attack at Central Coconut Research Station, Kayangulam (South India) was investigated during 1956. The disease was prevalent from May to October, when the humidity was above 70%, the temperature was about 27° C., and the sky was overcast with clouds for more than 50% of the time, throughout the period.

It is considered possible that during the S.W. Monsoon period on the West Coast of India, that epizootics of this disease may be initiated by artificial dissemination of the variety *rhinoceros* of *M. anisopliae* and thereby *O. rhinoceros* L. could be brought under control.

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Host Range and Transmission of the Pea Enation Mosaic Virus[1]

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Pea enation mosaic (PEM) is the most prevalent of the virus diseases affecting peas in western New York. Schroeder (1951) reported a 90% incidence of the disease in experimental plantings. While that figure represents an extreme value, a survey in commercial plantings in 1955 indicated a total viroses incidence in peas varying from 30 to 60%, 90% of which was PEM (McEwen & Schroeder 1956). A similar survey in 1956 showed that while PEM was almost absent from some areas, in others 75% or more of the plants were infected.

Pea plants infected with PEM virus are dwarfed and malformed but the degree of such symptoms varies. Leaf mottle is usually absent but chlorotic spots and flecks commonly occur. The best diagnostic symptom consists of ridges of proliferated tissue (enations) on the under surface of the foliage, especially along veins and bordering lucid flecks. Pods on infected plants bear enations and are frequently distorted and reduced in size. The disease studied here is believed identical to the pea mosaic of Osborn (1935) and of Synder (1934), the enation mosaic of Johnson & Jones (1937), and that caused by pea virus 1 of Stubbs (1937).

It was established by Osborn (1935) that the pea aphid (*Macrosiphum pisi* (Harris)) was an efficient vector of the PEM virus. His vector-virus studies indicated that the virus was of the persistent type (Watson & Roberts 1939). Some of the plant diseases caused by persistent viruses have, in several cases, been controlled through the use of insecticides to control the insect vector. More instances of successful control through this means are reported for leafhopper transmitted viruses, such as those inciting aster yellows of carrots and lettuce and curly top of sugar beets, than for those transmitted by aphids. However, instances in the latter category can be cited, particularly with reference to the potato leafroll virus.

Huckett (1945) reported that in small plot tests aphid control with rotenone reduced the incidence of mosaic in peas and increased the yield of pods. Although the exact pea mosaic involved was not specified in his report, the photograph of infected foliage included (fig. 6, A, B) indicated PEM. With the introduction of phosphate insecticides highly toxic to the pea aphid, it seemed probable that PEM could be controlled through an effective aphicidal program and an earlier report on progress in that connection was confirmatory (Swenson *et al.* 1954). Results in these tests, however, were inconsistent and available information did not explain these inconsistencies. In addition, published data on the host range of the PEM virus were inadequate to explain its distribution or its seasonal and regional variation in western New York. Experiments were therefore undertaken to determine the possible overwintering hosts of the PEM virus in this area and to re-examine the virus-vector relationship involved.

HOST RANGE.—Prior to the present investigation the host range of the PEM virus had been studied (Ainsworth 1940, Chaudhuri 1950, Hagedorn & Walker 1954, Johnson & Jones 1937, Osborn 1938, Pierce 1935, Simons 1954, Stubbs 1937). Of the reported susceptible species which might serve to overwinter the virus, only yellow sweetclover, alfalfa and alsike clover are common in the pea growing areas of New York. Of these three species, alfalfa and alsike clover were reported insusceptible by four of the five workers who tested them, and yellow sweetclover was reported as susceptible by only two of the four workers testing this species.

McEwen & Schroeder (1956) confirmed the susceptibility of alfalfa (*Medicago sativa* L.) and yellow sweetclover (*Melilotus officinalis* (L.) Lam.). In addition, Ladino clover (*Trifolium repens* L.), wild white clover (*T. repens* L.), white sweetclover (*Melilotus alba* Desr.), common vetch (*Vicia sativa* L.), and hairy vetch (*V. villosa* (Roth.)) were proved to be suscepts. Field observations prompted these workers to conclude that alfalfa, vetches and white clover were most important as sources of pri-

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mary inoculum of the PEM virus in western New York. Later research confirmed that alsike cover (*T. hybridum* L.), reported susceptible by Johnson & Jones (1937), could harbor the PEM virus but like alfalfa and white clover, symptoms are indistinct and transient. In these experiments it was observed that the above suscepts differed in their susceptibility to infection when exposed to viruliferous pea aphids. Also, the pea aphid recovered the virus more readily from some infected hosts than from others. The following experiments were carried out to determine the extent of these phenomena.

Young adult pea aphids were allowed to feed for 48 hours on a Bonneville pea plant infected with PEM. These aphids were then indexed in groups of 5 by allowing them to feed 24 hours on healthy pea plants. At the end of this time each group was transferred to a 3- to 4-week-old seedling of a plant species being tested. After aphids had fed 24 hours on each seedling they were killed by spraying with TEPP. If the indexing pea plant did not become infected, the corresponding seedling of the test species was discarded. Fifteen seedlings were tested in each plant species. All plants were held in a greenhouse at a temperature of 70 to 80° F. for about 3 weeks before attempts were made to recover the virus. With some plant species (common vetch, hairy vetch, crimson clover) visible symptoms served to distinguish infected from healthy plants, while in others (alfalfa, ladino clover, alsike clover, wild white clover, and to a lesser estent, yellow sweetclover and white sweetclover) distinct visible symptoms of infection were wanting. Plant infection was therefore determined by the ability of the pea aphid to infect young pea plants with PEM following an acquistion feeding on each inoculated test seedling. In such recovery attempts, each seedling subjected to viruliferous aphids 3 weeks earlier was colonized with 50 to 75 non-infective pea aphids. Forty-eight hours later, these aphids were transferred in groups of 5 to healthy pea plants in the 2- to 4-leaf-stage where they remained 48 hours before removal with TEPP spray. Plants were held in the greenhouse at 70 to 80° F. for 3 weeks before final readings on infection were recorded.

PEM virus was recovered from all or practically all of the seedlings of crimson clover, hairy vetch and common vetch previously exposed to aphids carrying this virus (table 1). However, according to this means of indexing, only 8 of the 64 alfalfa seedlings exposed to viruliferous aphids were infected. There was little difference in susceptibility among the five varieties, Grimm, Narragansett, Ranger, Kansas Common and Hairy Peruvian. Alsike clover, Ladino clover, wild white clover and yellow sweetclover showed a degree of susceptibility equal to that of alfalfa. White sweetclover was more susceptible than alfalfa but less so than the vetches and crimson clover.

Relative Accessibility of PEM Virus Among Suscepts.—Since the aforementioned experiment showed a variation among leguminous suscepts with regard to relative susceptibility to infection with the PEM virus by pea aphids, it seemed desirable to determine the relative accessibility of the virus in these different hosts to the aphid vector. For this determination, plants from the preceding experiment known to be carrying the PEM virus were utilized. One plant from each leguminous species, and from each variety of alfalfa, was colonized with 50 to 75 young adult non-viruliferous pea aphids for 48 hours. The aphids were then transferred, five per plant, to healthy pea plants in the 2- to 4-leaf-stage where they remained 48 hours before being killed with TEPP spray. The plants were then held in the greenhouse at 70 to 80° F. for 3 weeks and enation symptoms recorded. The results indicated that with the plants involved and techniques employed the relative accessibility of the virus to pea aphids closely paralleled the ease with which the plant species were infected by the vector carrying the PEM virus (table 1). Mechanical transmissions of PEM virus from the various legume hosts to pea using crude undiluted plant juice with 400 mesh carborundum as an abrasive showed that the virus was recovered from all of the hosts tested except alfalfa, alsike clover and Ladino clover (table 1). The conclusiveness of negative mechanical transfers is, however, questionable since relatively few pea plants were used in such attempts.

Table 1.—The relative susceptibility of various legumes to infection with pea enation mosaic virus by the pea aphid; the relative accessibility to this vector of the virus in these hosts and recovery by mechanical inoculation to peas.

Host Involved	Susceptibility of Seedlings: No. Infected/ No. Inoculated	Accessibility of Virus to Aphid Groups[a]	Recovery by Mechanical Inoculation[b]
Alfalfa var.			
Hairy Peruvian	2/12	3/15	−
Kansas Common	2/14	4/13	−
Grimm	1/13	1/7	−
Narragansett	1/13	1/7	−
Ranger	2/15	3/16	−
Alsike clover	2/14	2/15	−
Ladino clover	1/13	1/7	−
Wild white clover	2/15	3/14	+
Yellow sweetclover	2/11	1/9	+
White sweetclover	5/14	2/7	+
Crimson clover	14/15	7/7	+
Hairy vetch	13/13	6/6	+
Common vetch	13/14	7/8	+

[a] Aphids allowed to feed on seedling 48 hours, then tested in groups of five on healthy pea plants. Numerator = number of aphid groups infecting pea plant; denominator = number of aphid groups transferred from infected seedling to healthy pea plants.

[b] − = no recovery; + = positive transmission.

Transmission Studies.—Osborn (1935), Chaudhuri (1950) and Simons (1954) reported on the transmission of the PEM virus by the pea aphid and, with the exception of details concerning the latent period, data presented are in good agreement. It has been shown, however, that for many viruses, strains or isolates exist in nature which differ in pathogenicity (Holmes 1948, MacLachlan *et al.* 1953, Webb *et al.* 1952) and relative transmissibility by aphids (Bawden & Kassanis 1947). Smith (1931) reported that the green peach aphid required a period of 54 hours to effect transmission of the potato leafroll virus. Later reports (Kirkpatrick & Ross 1952, Day 1955) indicated much shorter periods necessary. Klostermeyer (1953) obtained transmission in 20 minutes. These reports plus those of Storey & Ryland (1955) and Stubbs (1955) showing that aphid colonies differed in their ability to transmit viruses prompted a study of the virus-vec-

tor relationships involved with the summer form of the pea aphid and an isolate of the PEM virus obtained locally.

Young adult pea aphids reared on healthy pea plants and starved 30 minutes were given acquisition feeding periods on PEM-infected pea plants of 2 minutes, 5 minutes, 30 minutes, 2 hours, 24 hours, and 48 hours. Healthy pea plants in the 2- to 4-leaf-stage were used as test plants to which these aphids were serially transferred, three per plant, and given test feedings of various durations. Ten replications were used for each feeding period tested. These plants were maintained in a greenhouse at 70 to 80° F. for 3 weeks and infections recorded. The results, summarized in table 2 and detailed for the 24-hour acquisition period in table 3, indicated that a feeding period of 30 minutes or less did not permit the aphid to acquire an infective quantity of virus. Following a 2-hour feeding period, 2 of the 10 groups of 3 aphids became infective but transmission was not accomplished until an additional period in excess of 24 hours had elapsed. Immediately after an acquisition feeding of 24 hours, 5 of the 10 aphid groups successfully transmitted the virus to test plants in a 5-minute feeding. However, transmission was not obtained with two of the 10 groups given the 24-hour acquisition feeding until more than 72 hours had elapsed (table 3). In this test, a 4-hour starvation period 72 hours after the aphids had been removed from the virus source had no appreciable affect on vector efficiency. An initial post-acquisition feeding starvation period of 4 hours duration following a 48-hour source feeding seemed detrimental. The data also showed that the five aphid groups which were immediately infective transmitted the virus to all of the plants on which they subsequently fed (table 3), whereas one group (group 7) which caused no infections during the first 72 hours failed to infect two of the plants to which it was later transferred.

In a second experiment non-infective young adult pea aphids were starved 30 minutes and given acquisition feeding periods of 4 and 8 hours after which they were serially transferred to healthy pea plants in the 2- to 4-leaf-stage and permitted various test feeding periods. Three aphids were used in each transfer with 10 replications for each feeding period tested. Results indicated that only 2 of the 10 groups of 3 aphids given a 4-hour acquisition feeding period were infective and that these were unable to transmit the virus until more than 23 hours had elapsed after the initiation of source plant feeding (table 2). Where an 8-hour acquisition feeding period was involved, 2 of the 10 groups transmitted the virus to plants during the first 16 hours, 3 more during the subsequent 28 hours and an additional 4 groups during the 44- to 92-hour period after they were removed from the source plant.

Simons (1954) reported that the PEM virus in burr clover became avalaible to the pea aphid several days before visible symptoms were expressed. Whether or not a similar phenomenon existed in the pea plant infected with this virus was determined in the following experiment. Non-infective young adult pea aphids were fed on an enation-infected pea plant for 48 hours. These were then transferred, 10 per plant, to each of three healthy pea plants in the 2- to 4-leaf-stage. After feeding 24 hours on these plants, the aphids were removed with TEPP spray and 24 hours later each of the three pea plants was colonized with 50 non-infective, young adult pea aphids. At 24-hour intervals thereafter, for 7 days, 20 aphids were removed from terminal foliage of each inoculated pea plant and placed, 5 per plant, on 4 healthy pea plants. The aphids were removed from these plants following a 48-hour infection feeding, the plants held in the greenhouse at 70 to 80° F. for 3 weeks and number of infected plants recorded. The experiment was repeated 2 weeks later.

The results of these experiments showed that the pea aphid was able to acquire an infective dosage of the PEM virus from one plant within 3 days after that plant was infested with viruliferous aphids (table 4). In 4 of the other plants a period of 4 days was required and 5 days elapsed before aphids from the other plant became infective. Visible symptom expression required 7 to 9 days.

Greenhouse experiments reported by Kerr (1954) led him to conclude that residues of three insecticides, one of which was parathion, were of little value in preventing viruliferous pea aphids from infecting broadbean plants with the PEM virus. Since this is of importance in control

Table 2.—Serial transmission of pea enation mosaic virus from pea to pea by the pea aphid with various acquisition and test feeding periods.[a]

Acquisition Feeding Time on Source	Infection Feeding Time on Test Plant in Series						No. Plants Infected of 10 Inoculated
	1	2	3	4	5	6	
Experiment 1							
2 min.	5 min.						0
		72 hrs.					0
5 min.	5 min.						0
		72 hrs.					0
30 min.	5 min.						0
		72 hrs.					0
2 hrs.	5 min.						0
		24 hrs.					0
			72 hrs.				2
24 hrs.	5 min.						5
		72 hrs.					8
			5 min.[b]				6
				15 min.			9
					30 min.		9
						48 hrs.	10
48 hrs.	5 min.[b]						3
		15 min.					3
			30 min.				4
				48 hrs.			9
Experiment 2							
4 hrs.	3 hrs.						0
		16 hrs.					0
			28 hrs.				2
8 hrs.	16 hrs.						2
		28 hrs.					5
			48 hrs.				9

[a] Three pea aphids per plant.
[b] Aphids starved 4 hours prior to feeding on test plant.

Table 3.—Serial transmission of pea enation mosaic virus from pea to pea by the pea aphid following a 24-hour acquisition feeding and various infection feeding periods.[a]

Infection Feeding Time on Test Plant	Inoculation Results in Plant Replicates									
	1	2	3	4	5	6	7	8	9	10
5 min.	+[b]	+	+	−	−	−	−	−	+	+
72 hrs.	+	+	+	+	+	+	−	−	+	+
5 min.[c]	+	+	+	−	−	−	+	−	+	+
15 min.	+	+	+	+	+	+	−	+	+	+
30 min.	+	+	+	+	+	+	−	+	+	+
48 hrs.	+	+	+	+	+	+	+	+	+	+

[a] Three pea aphids per plant.
[b] + indicates plant infected; − not infected.
[c] 4-hour starvation between the last feeding (72 hrs.) and this 5-minute feeding period on test plant.

Table 4.—Interval between infection feeding and acquisition of the pea enation mosaic virus by the pea aphid in each of six pea plants.

Interval in Days	Plant Number[a]					
	1	2	3	4	5	6
3	−	−	−	+	−	−
4	−	+	+	+	+	+
5	+	+	+	+	+	+
6	+	+	+	+	+	+
7	+	+	+	+[b]	+[b]	+
8	+[b]	+[b]	+	+	+	+
9	+	+	+[b]	+	+	+[b]

[a] + indicates aphids acquired the virus; − aphids did not acquire the virus.
[b] Visible symptoms.

practices, an experiment was conducted to determine the effect of a parathion residue on pea plants on the ability of viruliferous pea aphids to cause infection. Four-hundred young pea plants in the 4-leaf-stage were selected for uniformity and divided into groups of 200. One group was sprayed with parathion 1:3200 and the other group left unsprayed. Each group of plants was then subdivided into 4 groups of 50 each and used for daily inoculations. Commencing 24 hours after the plants were sprayed and continuing for 3 successive days, one group each of 50 sprayed and 50 unsprayed plants was colonized with 5 viruliferous pea aphids per plant. The aphids were allowed to feed 24 hours before removal with TEPP spray. Plants were then maintained in the greenhouse at 70 to 80° F. for 3 weeks before infections were recorded. The experiment was repeated 3 weeks later.

Results from these experiments indicated little effect of the parathion residue in reducing infection, even when the aphids were applied to foliage presenting parathion residue only 24 hours old (table 5).

Discussion.—The host range studies of PEM virus reported by McEwen & Schroeder (1956) have done much to explain the prevalence of this disease in canning peas in western New York. However, the wide variation in incidence of the disease lacked explanation as some of the hosts reported (*e.g.*, alfalfa and white clover) are widely distributed throughout pea growing areas in the state. The relative susceptibility of hosts to infection and the availability of the virus to the vectors involved may be major factors in virus distribution. Field observations lend weight to this premise. During 1955 and 1956, various species of vetches were commonly found infected with the PEM virus in western New York. Recovery attempts from these naturally infected plants as well as from plants experimentally infected in the greenhouse indicated that the virus was readily accessible to the pea aphid. Field observations also indicated that in many areas where the incidence of PEM in peas was high, vetches commonly occurred as weeds in ditch banks, fence rows and semi-abandoned fields. Such abundant distribution of vetches was seldom found in areas where the incidence of PEM in peas was low. In view of the close association of pea aphids with alfalfa and clovers (Davis 1915, Eichmann & Webster 1940, Evans & Gryisco 1956) the importance of these hosts as virus reservoirs should not be minimized. It may be that in some years vetches are more important reservoirs in western New York. Variations in the efficiency with which aphids can introduce and recover virus from different hosts are not unique to the PEM virus but the practical implications concerned with this particular virus warrant consideration.

The transmission phenomena observed in the present study vary somewhat from those previously reported. In contrast to results presented by Chaudhuri (1950) that a latent period of 4 to 20 hours existed regardless of duration of acquisition feeding, findings in the present study support those of Simons (1954) in which it was shown that provided the acquisition period was sufficiently prolonged, no additional latent period was necessary before transmission could be effected. This seems characteristic of all aphid-borne persistent viruses.

With burr clover as a host, Simons (1954) was unable to obtain infections with the PEM virus in feeding periods of less than 20 minutes duration while Kerr (1954), using broadbean as a host for this same virus, discounted the importance of feeding periods of 1 hour or less in causing infection. In the present study, infection feeding periods of 5 minutes were relatively effective and a 15-minute feeding period resulted in infection in almost all cases. Whether or not these contrasting data could be due to the host plant or aphid clone involved is not known.

PEM virus is one of the few persistent viruses readily transmitted by mechanical inoculation. As such it might be assumed that infection could be accomplished through superficial probing by the vector in the parenchymatous tissues rather than the necessity of reaching the phloem as suggested by Simons (1954). The high percentage of infections following 5-minute feedings in the tests reported here may support this assumption. However, Skotland & Hagedorn (1955) have shown through histological methods that the stylets of a percentage (18 of 69) of pea aphids allowed a 5-minute feeding period on peas do reach the phloem. These data indicate that the tissue penetration rate for the pea aphid is much more rapid than for other aphid species studied (Roberts 1940).

Watson & Roberts (1939) proposed that insect-transmitted virus diseases be classified as "persistent" and "non-persistent" and the following characteristics tend to define the former group:

(a) Vector, once infective, remains so for a considerable period, usually the remainder of its life.
(b) Transmission efficiency not affected by starving the vector prior to acquisition feeding.
(c) A latent period in the vector between the time the vector acquires an infective virus dosage and the time at which it can subsequently cause infection.

This classification serves a useful purpose in virus study but anomalous virus-vector relationships exist partic-

Table 5.—The relative ability of the pea aphid to transmit the pea enation mosaic virus to untreated and parathion-treated pea plant.[a]

Days After Spray Treatment	Number Plants Infected[b]	
	Parathion Sprayed	Unsprayed
1	72	84
2	78	84
3	80	88
4	84	92

[a] Five pea aphids per plant.
[b] Total of two replicates of 50 plants each.

ularly with respect to the latent period. Of about 15 aphid-borne phytopathogenic viruses normally considered persistent, at least three, beet yellows (Watson 1940), beet yellow net (Sylvester 1948) and leaf fleck of maize (Stoner 1952), have no sharply defined latent period. Studies on the potato leafroll virus indicate that a definite latent period may not be present (Klostermeyer 1953, Kirkpatrick & Ross 1954) although Day (1955) takes exception to such a view. Latent periods with leafhopper transmitted phytopathogenic viruses seem explicable on the basis of virus multiplication in the insect vector or the time necessary for a physical route of the virus in the vector prior to ejection in the salivary fluid. Transposition of these explanations to aphid transmitted persistent viruses seems unjustified with existing data. For such viruses, Kirkpatrick & Ross (1952) question the existence of a definite latent period while Day (1955) suggests virus multiplication in the aphid vector. Experiments on the PEM virus, reported here and elsewhere, indicate a definite latent period associated with the aphid transmission of this virus, although the minimum duration of that latent period has not been bracketed.

Experiments reported here agree with those of Kerr (1954) in demonstrating the ineffectiveness of parathion residue in preventing plant infection by viruliferous aphids. This would imply that residues of aphicides currently available cannot completely protect growing pea plants from infection due to migrant vectors carrying the PEM virus. Such insecticidal residue, however, will minimize the number of plants infected by a single aphid migrant and prevent the establishment of aphid colonies which, otherwise, would be available to spread secondary inocula 3 to 5 days after initial plant infection.

SUMMARY.—Greenhouse experiments showed that a number of annual and perennial legumes common in pea growing areas of New York were susceptible to the pea enation mosaic virus when inoculated by viruliferous pea aphids. In comparison with crimson clover, common vetch and hairy vetch which were readily infected, yellow sweetclover and white sweetclover were less susceptible and alfalfa, wild white clover, Ladino clover and alsike clover were infected in only a small percentage of trials. The availability of the virus to pea aphids in these various hosts closely paralleled the ease with which they were infected.

In the transmission of the pea enation mosaic virus from pea to pea with the pea aphid, the minimum acquisition feeding period was 2 hours, 5 minutes feeding was sufficient to produce infection, and the minimum total transmission period was 24 hours.

A parathion residue on pea plants was of insignificant value in protecting plants from infection by immediate feeding of viruliferous aphids.

Although visible symptoms of infection with pea enation mosaic required 7 to 9 days in the pea plant, the virus was readily available to pea aphids 3 to 5 days after initial infection.

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The Relative Effectiveness of Various Insecticide-Fungicide Seed Treatments on Lima Beans After Several Months Storage[1]

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The seed-corn maggot, *Hylemya cilicrura* (Rond.), and seed decay fungi attacking the seeds of large seeded vegetables and corn in New York are effectively controlled by a combination insecticide-fungicide seed treatment. This method has also proved successful for the control of these and similar pest complexes in other vegetable growing districts of the United States. In consequence insecticide-fungicide seed treatments are now in fairly general use.

The desirability of treating seed several months prior to planting was early recognized (Howe *et al.* 1952). Data pertaining to the effectiveness of such seed coatings after several months' storage, however, are limited and somewhat contradictory. Howe *et al.* (1952) reported that 8 months' storage of seed of Clark's Bush lima beans treated with thiram (1 ounce per bushel) and lindane (0.25 ounce per bushel) had no adverse effect on pesticidal properties or on the seed. In a continuation of these storage tests, Natti & Schroeder (1955) reported that sufficient pesticidal effect of the lindane-thiram treatment persisted to provide good protection after 32 months' storage. Ditman *et al.* (1955) reported that seed of wrinkled pea (Alaska) treated with 0.25 ounce of lindane per bushel became heavily infested with seed-corn maggots when planted 12 months after treating. In comparison with seed not treated with lindane, the infestation in the treated seed developed later but at 17 days after planting lindane-treated and untreated seed were about equally infested.

In tests on field corn, Gould (1955) found that a high dosage of lindane or BHC as a seed treatment caused plant distortion. Following storage, however, this effect was reduced. Corn treated with lindane (4 ounces per 100 pounds) showed 40% distortion when planted immediately after treatment, whereas only 1% of the plants produced were distorted when the treated seed had been stored 24 months before use. These results were interpreted as a dissipation of lindane during storage. More recently, Nugent & Hofmaster (1957) reported on tests with seed of snap beans, sweet corn and Fordhook lima beans treated with captan or thiram in combination with aldrin, dieldrin, heptachlor or lindane. They found no evidence of injury to the seed or loss of insect or disease control following 3 months in the treated condition in commercial storage. In another experiment dieldrin retained its effectiveness better than aldrin, heptachlor or lindane when treated snap bean seed was held a year under laboratory conditions. This seed according to these workers was not injured by the treatment but the insecticidal activity of the compounds decreased.

In view of the fact that published data and statements on the retention of insecticidal properties of treated seed are inconclusive, tests were initiated to obtain further information on this point. Results obtained with thiram and three insecticides are presented.

Materials and Methods.—A single lot of Fordhook 242 lima beans was used for all treatments. This sample was screened to remove large and small seeds and provide a uniform seed size for test purposes. The seeds thus obtanied weighed approximately 1 pound per 500 seeds. Seed was treated by the slurry method (Howe *et al.* 1952) using 2% Methocel as a sticker. The slurry was applied at the rate of ½ pint per bushel of seed. The fungicide thiram (from Arasan SF-X) was used throughout the tests. It was combined in various proportions with wettable powders of 25% Diazinon and 75% dieldrin and lindane. In treating, the pesticides were combined and mixed dry before incorporation in the Methocel preparation. A measured amount of the Methocel-pesticide mixture was then added to each weighed seed lot in a glass jar. Seed coverage was obtained by slowly rotating the jar and contents on a motor-driven wheel for 3 minutes. Preliminary trials using graphite had indicated that this method provided a uniform coverage of the seed.

The seed was planted in the field by means of a Planet Jr. seeder. The plate was removed from the seeder to permit the ready flow of seed through the opening at the lower end of the seeder box. As one operator pushed the seeder along the row, another hand-fed the seed in such a manner that the seed was uniformly distributed. A randomized block design with six replications of each treatment was used. Each replicate consisted of a single

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row 10 to 12 feet long in which 50 seeds were planted. Digester tankage was spread over each row after planting to provide an attractive oviposition site for adults of the seed-corn maggot. In the 1955 planting only a small amount of tankage was available and this was broadcast over the test area. In the 1956 planting the tankage was spread with the Planet Jr. seeder which delivered a narrow band over each row at the rate of approximately 1 pound per 100 linear feet. The effectiveness of the treatments was determined by counts on the total emergence and number of maggot-injured plants. These counts were made as soon as the plants had two fully expanded leaves.

RESULTS.—*Immediate effectiveness.*—The experiment consisted of 39 treatments. Each of the insecticides Diazinon, dieldrin and lindane was tested at 0.125 and 0.25 ounce per bushel separately and in combination with thiram at 1, 2 and 4 ounces. In addition thiram was tested by itself at each dosage. A check which received no treatment and one which received Methocel alone were also included. The seed was treated September 7, 1955 and planted in sandy soil September 9, 1955. With abundant moisture and ensuing warm weather, germination was rapid. Treatments containing insecticide and fungicide, regardless of rate or kind of insecticide, had stands of approximately 90%. A similary good stand was obtained where the fungicide was used alone. Stand obtained following no treatment, treatment with Methocel alone or treatment with dieldrin or lindane by themselves at either rate was approximately 70%. Treatment with Diazinon by itself, at either rate, reduced emergence nearly 50%. In this planting, a considerable amount of maggot injury occurred in local areas. This spotty maggot infestation was attributed to poor distribution of tankage (broadcast treatment) and was so pronounced as to invalidate control data. The association of maggot attack with areas receiving a recognizable deposit of tankage was easily observed. It was similarly observed that plants from insecticide-treated seed were free from maggot injury even in these local areas of severe attack.

Effectiveness After 8 Months' Storage.—A portion of the seed treated September 7, 1955 was stored in cloth bags in an unheated barn until the following spring. This seed was planted early in May in sandy soil using the method of planting previously described. A duplicate planting of freshly treated seed was made 3 days earlier in an adjacent part of the field. Apparently soil temperature and moisture conditions at this time of planting were extremely critical, for while a satisfactory stand was obtained in the planting of stored-treated seeds less than 10% of the plants emerged in the other planting. Consequently, it was not possible to make a direct comparison between freshly-treated and stored-treated seed.

Data obtained on the total emergence and number of maggot-injured plants from the stored-treated seed are presented in table 1. Very few plants emerged from seed not receiving thiram treatment and approximately 55% of the plants from seed not receiving insecticide were injured by the seed-corn maggot. A factorial analysis of the

Table 1.—Emergence of Fordhook 242 lima beans and number of plants injured by the seed-corn maggot following treatment with various dosages of thiram and insecticides 8 months before planting.

Treatment			Total Emergence[a]	No. Maggot-Injured Plants[b]
Thiram (Oz./Bu.)	Insecticide	Dosage (Oz./Bu.)		
0	Diazinon	0.125	6	1
1	Diazinon	.125	33	2
2	Diazinon	.125	34	3
4	Diazinon	.125	36	2
0	Diazinon	.25	3	0
1	Diazinon	.25	32	4
2	Diazinon	.25	36	2
4	Diazinon	.25	38	1
0	Dieldrin	.125	7	0
1	Dieldrin	.125	33	5
2	Dieldrin	.125	34	8
4	Dieldrin	.125	36	5
0	Dieldrin	.25	7	1
1	Dieldrin	.25	32	4
2	Dieldrin	.25	38	4
4	Dieldrin	.25	36	3
0	Lindane	.125	8	2
1	Lindane	.125	34	10
2	Lindane	.125	36	11
4	Lindane	.125	35	8
0	Lindane	.25	7	2
1	Lindane	.25	30	4
2	Lindane	.25	33	3
4	Lindane	.25	37	6
0	Methocel only	None	9	6
1	No insecticide		30	18
2	No insecticide		32	19
4	No insecticide		32	15
Check	Untreated seed		8	5

[a] From 50 seeds planted. Mean of six replicates.
[b] Of the total emergence.

Table 2.—Summary of significant treatment effects.

A. Mean emergence at various rates of thiram in combination with insecticides.

Thiram (Oz./Bu.)	Total Emergence[a]
1	32.4
2	35.0
4	36.4

L.S.D. 19:1—2.1; 99:1—2.8

B. Mean number of maggot-injured plants with three insecticides in combination with thiram.

Insecticide	No. Maggot-Injured Plants[b]
Diazinon	2.4
Dieldrin	4.7
Lindane	7.1

L.S.D. 19:1—1.5; 99:1—2.0

C. Mean number of maggot-injured plants at two dosages of three insecticides in combination with thiram.

Insecticide (Oz./Bu.)	No. Maggot-Injured Plants[b]
0.125	6.2
0.25	3.3

L.S.D. 19:1—1.3; 99:1—1.8

[a] From 50 seeds planted. Mean of 36 replicates.
[b] From 50 seeds planted. Mean of 54 replicates.

insecticide-fungicide combination treatments showed a significant effect of the thiram dosage on total emergence (table 2-A). The magnitude of this increased emergence following increased dosage of thiram was not sufficient, however, to be of practical importance. A similar analysis showed that maggot control from all treatments containing Diazinon was significantly greater than that from all treatments containing dieldrin which in turn was more effective than lindane (table 2-B). Maggot control with the insecticides at 0.25 ounce per bushel was significantly greater than that obtained with one-half this dosage (table 2-C). A significant interaction of insecticides × insecticide rates was also demonstrated. This latter interaction is readily discernible for while maggot control was much improved by increasing the dosage of lindane or dieldrin from 0.125 to 0.25 ounce per bushel, control with Diazinon was equally good at either dosage.

DISCUSSION.—Although directly comparable evaluations of freshly treated and stored-treated seed are not possible from the 1956 tests, a comparison with earlier work seems warranted. In a separate experiment conducted in 1955, the same seed lot and treatments were used as those employed in the test reported herein. In that experiment 67.2% of the plants from untreated seed showed injury by the seed-corn maggot (McEwen *et al.* 1955). Similarly injured plants from seed treated with Diazinon at 0.25, 0.125 and 0.062 ounce per bushel amounted to 1.3, 1.4 and 3.9%, respectively. Comparative injury figures for dieldrin were 2.6, 2.6 and 3.8, and for lindane were 1.4, 1.4 and 7.8. Since a greater percentage of plants from untreated seed was injured in the 1955 planting (67.2%) than in the storage test of 1956 (55%), it would be expected that maggot injury following a given treatment would be slightly less in the latter. Seed treated with dieldrin or lindane at 0.125 ounce per bushel in the 1955 planting was well protected from maggot injury (less than 3% of the plants injured) while following 8 months' storage identical treatments permitted injury to 18% (dieldrin) and 28% (lindane) of the plants (table 3). Where the dosage rate was 0.25 ounce per bushel the loss of effectiveness of the seed treatment for maggot control was less evident. From the 1955 test, it is apparent that when lima bean seed is treated with dieldrin or lindane at the rate of 0.25 ounce per bushel 50 to 75% of this dosage can be lost without noticeable effect on maggot control. This may explain earlier reports (Howe *et al.* 1952, Natti & Schroeder 1955) that the pesticidal properties of treated seed were not lost during prolonged storage. Of the treatments tested, those containing Diazinon retained effectiveness against seed-corn maggot attack better than those containing dieldrin or lindane. Although maggot injury following lindane treatments was slightly more, after 8 months' storage, than that with dieldrin-treated seed similarly stored, the two insecticides were esentially comparable in effectiveness at the 0.25 ounce per bushel dosage.

Table 3.—Emerged plants of Fordhook 242 lima beans injured by the seed-corn maggot following seed treatment with each of three insecticides at two rates in combination with thiram 8 months before planting.

INSECTICIDE	INSECTICIDE (OZ./BU.)	PER CENT OF PLANTS INJURED[a]
Diazinon	0.125	7
	.25	7
Dieldrin	.125	18
	.25	10
Lindane	.125	28
	.25	13

[a] Based on counts of 18 replicates of approximately 34 plants each.

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Some Factors Influencing the Predation of *Polistes* Wasps on the Tobacco Hornworm[1]

R. L. Rabb and F. R. Lawson[2]

Wasps of the genus *Polistes* have, on the basis of general field observations, been recognized for many years as important natural enemies of the tobacco hornworm, *Protoparce sexta* (Johan.), and the tomato hornworm, *P. quinquemaculata* (Haw.) (Gilmore 1938, Madden & Chamberlin 1945, Rabb 1953). They have also been observed to prey upon other species of free-feeding larvae, chiefly caterpillars (Ballou 1915, Quaintance & Brues 1905, Hixson 1941, Hinds & Dew 1915). In recent years Lawson & Rabb have accumulated data which strongly indicate that *Polistes* wasps are the dominant natural enemies of hornworms in North Carolina and that the degree of their activities in tobacco in many cases determines the severity of hornworm damage. They found that the wasps will nest in wooden shelters and can be moved in them to desired locations. The use of such shelters around tobacco fields brought about a reduction in hornworm numbers and in many cases prevented economic loss of leaves (Lawson *et al.* unpublished).

Further progress in the utilization of *Polistes* wasps or the evaluation of their effectiveness is dependent upon the acquisition of a more basic understanding of their biology and particularly of the factors influencing their effectiveness against hornworms. The studies reported here were concerned primarily with the effect of distance between *Polistes* colonies and tobacco fields and the presence of other prey species in the vicinity on the number of wasps procuring prey from tobacco fields.

Muesebeck *et al.* (1951) and Rabb (1950) concur in listing the following *Polistes* species from North Carolina: *P. annularis* (Linnaeus), *P. exclamans exclamans* Viereck, *P. fuscatus fuscatus* (Fabricius), *P. hunteri hunteri* Bequaert, *P. metricus* Say, and *P. rubiginosus* Lepeletier. In addition, Muesebeck *et al.* (1951) report *P. fuscatus pallipes* from the mountains and Rabb (1950) records *P. bellicosus* Cresson from the coastal area of this state. Bequaert (1940) and Bohart (1949) treat the North American *Polistes* taxonomically.

In general, the life and seasonal histories of these species are quite similar. In the spring, overwintering females (queens) begin construction of nests, each consisting of a single comb and constructed of fiber collected from many sources, such as dead trees, weeds, or lumber. The number of cells constructed prior to emergence of the workers varies considerably and is dependent in large measure upon the number of overwintering females founding each colony. Generally, single queen nests consist of 10 to 15 cells before emergence of the workers. Trees, shrubs, building, and many other situations are utilized as nesting sites.

One egg is deposted in each cell and the wasp larvae are fed honey-like materials, insect fragments, and water by the adults. Mature larvae spin caps to their cells and pupate. During early spring about 2 months are required for the life cycle; however, this period is shorter during the summer months. Generally, wasps emerging before late summer are females which participate in nest construction, food procurement and larval care. Males do not normally appear until late summer or fall and take no active part in the work of the colony. At the end of the season, nests of *fuscatus* and *exclamans* vary from a few dozen to about 450 cells. Some *annularis* nests contain over 1000 cells.

In procuring prey the female does not utilize her sting; instead, she kills the prey with her mandibles. Small prey may be rolled into a ball and carried almost intact to the nest. When larger prey, such as third- to fifth-instar hornworms, are killed, only a portion of each carcass is carried to the nest at one time, and a wasp may return to the carcass for additional loads. The adults ingest much of the body fluids of their prey in fashioning it into balls of meat of suitable dimensions for transporting.

Methods.—In order to obtain wasp colonies, wasp shelters were placed along fence rows, field borders, and drainage ditches in March, 1956. These shelters, similar to those utilized by Lawson *et al.* (no date), were wooden boxes 8″×8″×11″, with the bottoms open but screened with chicken wire to prevent birds from reaching the nests. Each shelter was constructed so that the open bottom could easily be closed with a detachable door. They were nailed to posts at a height of from 3 to 4 feet. *Polistes* nested in many of these by June. In May and early June, 10 colonies each of *exclamans* and *fuscatus* were moved to each of three stations on the Central Crops Research Farm, Clayton, N. C. The shelters were closed on the night prior to moving. The location of each station relative to tobacco and other vegetation is shown in figure 1. Hornworm populations on tobacco in the experimental area were kept at a very low level by insecticidal applications except in fields C9a and B8 (fig. 1). Field B8 was as far or farther from the stations of *Polistes* colonies than field C9a.

Colonies of each species were segregated at each station. These colonies were 15 to 40 feet from tobacco at station 1, 400 feet at station 2, and 800 feet at station 3. Since some colonies failed to develop normally, additional ones were moved to the stations from time to time to keep the wasp populations of the two species and of the various stations roughly comparable. Station 4 was established about 400 feet from tobacco during the period from July 24 to 30. Most of the colonies at this station were moved from Whiteville, a distance of about 120 miles from Clayton.

At invervals during June, July, and August, wasps carrying prey were captured in nets and robbed when they returned to their nests. The prey were preserved in kero-

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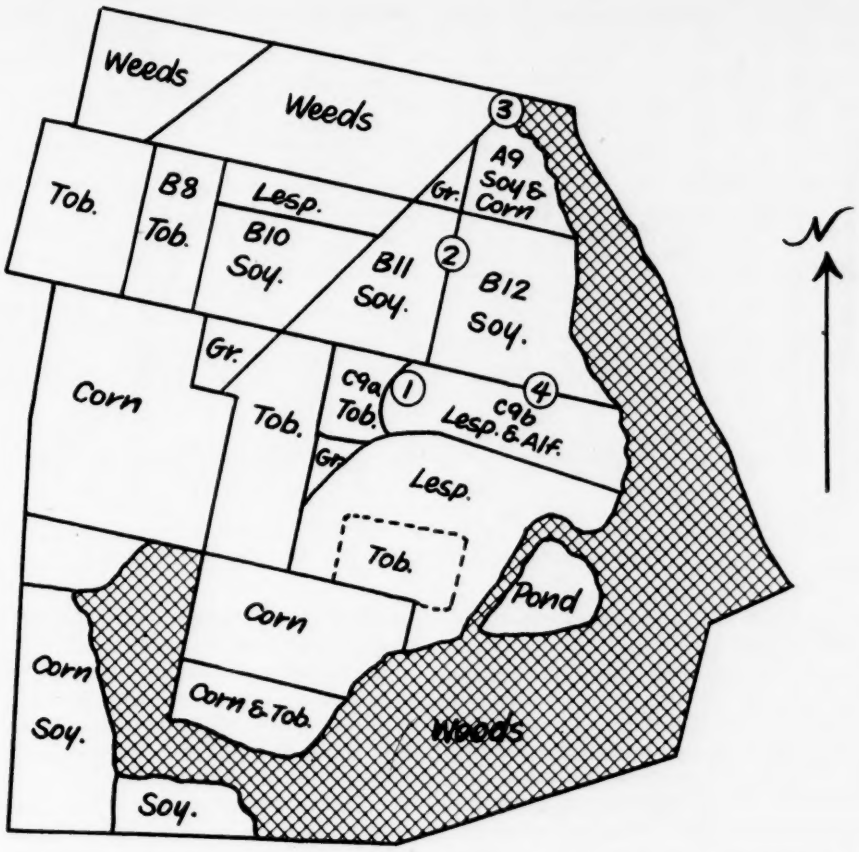

FIG. 1.—Map of the eastern portion of the Central Crops Research Station showing the location of batteries of *Polistes* colonies and adjacent crops. Clayton, N. C., 1956. (Alf. = alfalfa; gr. = grass; lesp. = lespedeza; soy. = soybeans; tob. = tobacco; encircled numbers 1–4 = stations of *Polistes* colonies.)

sene-alcohol-acetic acid-dioxan (Peterson 1948) and later identified by comparison with known material. The larval characteristics of most significance in these determinations are given by Peterson (1948).

Hornworm populations and mortality were determined by counts of eggs and larvae made in a field of tobacco (C9a in fig. 1) which was not treated with insecticides. Twenty-five marked plants were examined daily or on alternate days (Sundays excepted) and records of the occurrence, mortality, and survival of eggs and larvae were made as described by Lawson & Rabb. In order to segregate larvae on marked plants from those on adjacent plants, a metal collar about 8 inches high was placed about the base of each marked plant, as soon as it became infested. A general impression of the seasonal trend in populations of other species of prey was obtained by field observations and collections.

Another experiment, designed primarily to estimate *Polistes* populations, provided supplementary data on the proportions of wasps from each station hunting in the tobacco field (C9a). Wasps were netted in the tobacco field, each was marked on the top of the thorax with a spot of quick-drying lacquer and released. The marked and unmarked wasps on the nests at the various stations were counted at night.

RESULTS AND DISCUSSION.—*Seasonal variations in populations of wasps and prey.*—Since emphasis here is placed upon the predations of *Polistes* on the tobacco hornworm, seasonal variation in populations of wasps and prey are described in reference to the occurrence of hornworm broods. Three broods occur: the first in May and June, the second in July and August, and the third in September and October (Lawson & Rabb). Only the first two broods are of direct concern to most tobacco growers since the third brood occurs principally on suckers after harvest. The seasonal occurrence and size of the first two hornworm broods in field C9a (fig. 1) are shown in figure 2.

Comparisons of wasp populations and rates of prey collections during the first two hornworm broods are made in table 1. A small portion of the colonies designated as *P. f. fuscatus* (F.) were actually *P. metricus* Say. These two species are difficult to separate when material consists of small colonies and live specimens, as was the case when the colonies were moved to the stations. The data indicate about a three-fold increase in wasp populations and rate of predation between late June and early August.

The identifications of prey collected from *P. e. exclamans* and *P. f. fuscatus* are shown in table 2. Little difference in the prey composition of the two predators is indicated when the data from all stations are considered collectively. Over 90% of the prey consisted of lepidop-

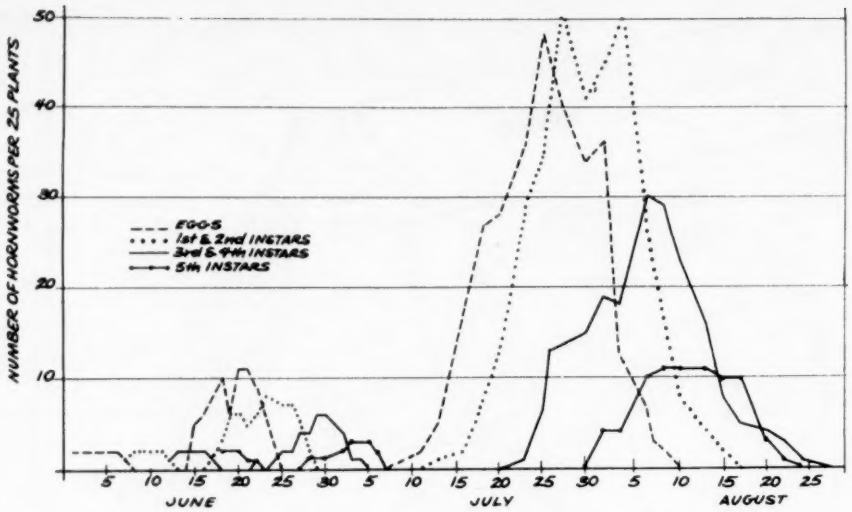

FIG. 2.—Seasonal occurrence of eggs and larvae of the first and second broods of tobacco hornworms in field C9a, Clayton, N. C., 1956.

terous larvae, most of which feed in exposed situations on foliage and principally on herbaceous plants.

The extent of predation on selected prey species, including the most commonly taken by the wasps, during the latter part of the first brood, between the first and second broods, and during the second brood of hornworms is shown in table 3. The seasonal occurrence of hornworms (fig. 2) is reflected in the prey collections. *Protoparce* comprised an appreciable portion of the prey during but not between broods.

Both species of wasps took more prey from wooded areas during late June and early July than during late July and early August, in spite of the fact that general collections indicated an increase in free-feeding larvae on trees as the season advanced. Among the tree-infesting species which were more numerous in the June than in the July and August samples of prey, were the white-marked tussock moth, *Hemerocampa leucostigma* (J. E. Smith), and certain notodontids and arctiids. A large portion of the latter were probably the fall webworm, *Hyphantria cunea* (Drury), which occurred in abundance on certain of the deciduous trees and shrubs bordering the crop land.

Heliothis larvae rarely fell prey to *Polistes* in late June but were taken in larger numbers as the season advanced. This change seems to have been influenced by the seasonal change in the feeding habits of *Heliothis* larvae. During May, June, and early July, tobacco budworms (*Heliothis virescens* (F.)) occur mostly inside the terminal buds of tobacco and corn earworms (*H. zea* (Boddie)) in the whorls of corn plants or inside the shucks of corn ears where they are protected from the wasps. In July, however, after the terminal buds of tobacco had produced floral heads, *Heliothis* larvae were observed to become abundant in exposed situations on tobacco leaves, suckers, and floral parts. In these situations, they were readily taken by both species of wasps, as can be seen from the prey samples taken during July and August (table 3).

In June, the green cloverworm (*Plathypena scabra* (F.)) did not occur in the prey samples taken from either species of predator, whereas it comprised 26 and 30%, respectively, of the prey taken from *exclamans* and *fuscatus* during late July and early August. Soybeans, the chief host of the green cloverworm in the experimental area, did not become heavily infested with this larva until about the middle of July.

Variations in prey as influenced by colony location.—Variations in the percentages of tobacco hornworms and green cloverworms in the prey samples were used to study the effects of colony location. These were the two most numerous species in the samples, and their field populations were fairly well defined in terms of location and size. Prey collections made during the second brood were chosen for statistical treatment because they were larger and more uniform than those taken earlier. In arranging the data for analysis, collections on different days were treated as replications (table 4).

Data from the first three stations show that the percentage of hornworms in the prey of *exclamans* decreased with distance from tobacco. Roughly five times as many hornworms were taken at station 1 as at station 3,800 feet away. This same species brought in fewer green cloverworms at station 1 than at the other stations. Other

Table 1.—Estimates of wasp populations and numbers of wasps per hour bringing in prey at stations 1, 2, and 3, collectively, during the first and second broods of hornworms. Clayton, N. C., 1956.

Hornworm Brood	Species of *Polistes*	No. of Colonies	Estimated No. Female Wasps	Man-Hours Collecting Prey	Number of Prey/Hr.	
					Collected	Missed[a]
First[b]	*exclamans*	45	229	21	4.8	1.3
	fuscatus	44	136	27	3.8	1.7
Second[c]	*exclamans*	45	756	27	16.0	6.2
	fuscatus	51	476	35	8.1	2.8

[a] Estimated.
[b] Collections made from June 27 through July 6. Colony populations estimated on July 5.
[c] Collections made from July 23 through August 14. Colony populations estimated on July 27.

Table 2.—Prey collected from *Polistes e. exclamans* and *P. f. fuscatus*. Clayton, N. C., 1956.

Species Identified in Prey Samples	Percentage of Each Kind of Prey Identified in Samples[a]	
	exclamans	*fuscatus*
Lepidoptera		
Arctiidae spp.	4.0	4.7
Hesperiidae spp.	0.1	0.7
Geometridae spp.	0.5	0.1
Liparidae		
Hemerocampa leucostigma	1.1	2.5
Notodontidae spp.	0.9	0.3
Nymphalidae		
Precis coenis (Hbn.)	0.0	0.7
Phalaenidae		
Acronicta sp.	0.2	0.1
Catocala sp.	0.4	0.1
Heliothis virescens	5.8	2.5
Heliothis zea	0.9	0.7
Heliothis spp.[b]	5.0	6.0
Plathypena scabra	22.0	24.0
Prodenia sp.	0.5	1.5
Undetermined	10.0	12.7
Pieridae		
Colias philodice eurytheme Bdv.	0.9	1.5
Undetermined	0.1	0.3
Pyralidae		
Loxostege sp.	0.1	0.7
Sphingidae		
Protoparce sexta (Johan.)	22.0	22.0
Protoparce spp.[c]	4.5	0.9
Undetermined	2.0	0.3
Undetermined Lepidoptera	8.0	8.2
Coleoptera		
Leptinotarsa decemlineata (Say)	2.7	0.0
Other Arthropoda	4.1	6.0
Unidentified animal matter	1.2	0.7

[a] 720 prey specimens from *P. e. exclamans* and 511 from *P. f. fuscatus*.
[b] Characters on specimens not sufficient for distinguishing between *H. virescens* and *H. zea*, the only *Heliothis* spp. found in the area.
[c] Mostly second-instar larvae which could possibly have been either *P. quinquemaculata* or *P. sexta* but which were probably the latter.

examples of the effect of colony location on the prey of *exclamans* were apparent in data collected. More tobacco budworms were collected from wasps at station 1 than at stations further removed from tobacco. In addition, wasps brought in more larvae of Colorado potato beetle at stations 2 and 3 than at the other two stations. The only potato beetle larvae observed in the experimental area were on a solanaceous weed in field A9, between stations 2 and 3.

Table 4.—Percentages of *Protoparce sexta* and *Plathypena scabra* in prey of *Polistes* nesting at various distances from tobacco. Clayton, N. C., 1956.

Station No.	Feet from Tobacco	Prey of *P. exclamans*			Prey of *P. fuscatus*		
		Sample Size[a]	Per Cent		Sample Size[b]	Per Cent	
			sexta	*scabra*		*sexta*	*scabra*
1	40	94	56	12	104	26	23
2	400	98	36	37	113	40	27
3	800	99	10	44	100	24	23
4	400	93	6	47	86	8[c]	39
L.S.D. at 5% level			15	23			

[a] Total of 4 replications.
[b] Total of 3 replications.
[c] Significantly lower than the mean of other stations. This comparison justified in spite of non-significant F-test, because wasps were moved to station 4 about 2 months later than at other stations.

In contrast to the gradient shown by *exclamans*, *fuscatus* caught nearly the same proportions of hornworms and green cloverworms at stations 1, 2, and 3. This species is slightly larger than *exclamans* and seems to fly further from the nests in search of prey.

Both species of wasps at station 4 took fewer hornworms than at station 2, even though both stations were the same distance from tobacco. In addition, neither species took more hornworms at station 4 than at station 3, although station 3 was more than twice as far from hornworm infestations as station 4. Field observations of marked wasps have shown that there is a tendency for *Polistes* to return to a habitat in which they have previously found prey. If these observations are representative, then wasps of each colony develop hunting patterns which probably change slowly as the populations of prey in their hunting habitats change and as newly emerged females are added to the populations. Therefore, the hunting patterns of wasps at stations 1 to 3 might be expected to be different from those of wasps at station 4, which was established much later in the season than the other stations. Wasps at station 4 were moved in when prey was abundant in legumes close to the shelters and thus may not have developed the habit of hunting in the more distant tobacco fields.

The numbers of wasps marked in the tobacco field C9a

Table 3.—The percentages of certain species in the prey of *Polistes* during and between the first and second broods of *Protoparce sexta*. Near Clayton, N. C., 1956.

Species of Prey	Habitat[a]	Percentage of Total Prey During and Between Broods[b]					
		First Brood (Late)		Between Broods		Second Brood	
		exclamans	*fuscatus*	*exclamans*	*fuscatus*	*exclamans*	*fuscatus*
Protoparce spp.	H	9	28	0	0	31	26
Heliothis spp.	H	2	2	6	7	13	11
Plathypena scabra	H	0	0	2	7	26	30
Arctiidae spp	T	20	26	21	14	1	1
Notodontidae spp.	T	7	2	6	2	0	0
Hemerocampa leucostigma	T	11	12	4	11	0	0.3
Sample Size		52	47	52	57	613	407

[a] H, herbaceous vegetation; T, trees and shrubs.
[b] Prey collections were made June 27–29 for 1st brood, July 3–6 for between broods, and July 23–August 14 for 2nd brood.

Table 5.—The number of *Polistes* marked in a tobacco field and recovered on nests at various distances. Clayton, N. C., 1956

Date of Marking	No. Wasps Marked	Date Nests Examined	Station No.	Distance (Ft.) from Marking Point	No. Observed			Per Cent of All Marked Wasps Found on Nest
					Nests	Total Wasps	Marked Wasps	
				Polistes e. exclamans				
July 20–27	32	July 27	1	40	14	132[a]	12	38
			2	400	16	174	1	3
			3	800	15	151	0	0
Aug. 13–14	89	Aug 14	1	40	14	358[b]	33	37
			2	400	13	—	3	3
			3	800	14	—	2	2
			4	388	10	—	6	7
				Polistes f. fuscatus				
July 20–27	25	July 27	1	15	12	85[a]	7	28
			2	400	12	52	1	4
			3	800	12	38	0	0
Aug. 13–14	102	Aug. 14	1	15	12	241[b]	18	18
			2	400	7	—	4	4
			3	800	8	—	1	1

[a] Only wasps which could be fully observed were recorded. The estimated populations of nests at the three stations were 187, 312, and 257 wasps, respectively, for *P. exclamans* and 133, 119, and 128 for *P. fuscatus*.

[b] This represents the total number at station 1, since the wasps were anaesthetized to make a complete examination possible. At the other stations, only the marked wasps on the face of the comb were counted.

and later recovered on the nests at the various stations are shown in table 5. It is clear that a high proportion of the wasps recovered were found at station 1. Very few marked wasps were seen at stations 2 and 3. So great a difference would hardly be expected from the prey data (table 4), which show that appreciable numbers of hornworms were taken at the more distant stations. This apparent inconsistency is undoubtedly due to a difference in the population sampled by the two techniques. The marking technique involved only wasps hunting in a single field (C9a) whereas the prey collections involved all of the wasps at each station. Many of the hornworms in the prey samples may have been caught in field B8, which was heavily infested, or in other tobacco fields. The higher proportion of hornworms taken at the distant stations by *fuscatus* may have been the result of the greater hunting range shown by this species.

Effects of cultural practices on the composition of Polistes prey.—Cultural practices or other factors which affect host plants of prey species would be expected to cause variations in the prey of *Polistes*. For example, the soybeans in fields B10, B11, and B12 (fig. 1) were plowed under on August 10. On August 9, populations of green cloverworms were 48 larvae per 300 sweeps on soybeans in B12 and 36 on lespedeza in C9b. The percentages of cloverworms and hornworms in samples of *P. exclamans* prey the day before and 3 days after the soybeans were plowed under are shown in table 6. The percentage of cloverworms in the samples dropped at all stations after plowing. The smaller decrease at stations 1 and 4 may have been due to the presence of cloverworms on lespedeza in field C9b, adjacent to these two stations (fig. 1). Changes in the percentages of hornworms following plowing of the soybeans were not consistent. It would be logical to expect that a decrease in the take of one prey species would be accompanied by an increase in the take of alternate prey. The failure of these samples to show such an increase in hornworms may have been due to the seasonal drop in the population of third- and fourth-instar hornworms between August 9 and 13 (fig. 2).

Instars of hornworms taken by Polistes.—Madden & Chamberlin (1945) found that the widths of the head capsules of the five instars of *Protoparce sexta* are in agreement with Dyar's rule (1890), since the increase from first- to fifth-instar follows a geometric progression rather closely. In determining the instars of hornworms taken from *Polistes*, however, head capsule measurements were of limited value, since head capsules were mutilated or missing from many prey specimens carried to the nests by wasps. In practically all cases however, spiracles were present. Therefore, measurements of spiracles of the various instars were made and it was found that they could be used in determination of instars. An appreciable number of the larvae in the prey samples were in the process of moulting; therefore, such individuals were arbitrarily recorded as the smaller of the two instars involved. It was not possible to distinguish between *Protoparce sexta* and *P. quinquemaculata* in the first- and second-instars, however the number of *P. quinquemaculata* of the later instars was found to be negligible both in prey samples and in tobacco fields.

Table 6.—The percentages of *Plathypena scabra* and *Protoparce sexta* in prey samples taken from *Polistes e. exclamans* before (August 9) and after (August 13) plowing under soybeans near the wasp colonies. Clayton, N. C., 1956

Station No.	Per Cent *Plathypena* in Prey		Per Cent *Protoparce* in Prey	
	Before Plowing	After Plowing	Before Plowing	After Plowing
1	11	4	44	40
2	42	12	42	23
3	62	21	8	13
4	27	19	12	37

No first-instars and relatively few second- and fifth-instars were observed in the prey samples. However, the seasonal occurrence and abundance of instars 2 to 5 were reflected to some extent in the prey samples. For example, the number of each instar of *Protoparce* in prey samples taken at two different times during the second brood of hornworms is shown in table 7. In the prey collected from July 23 to 26, there was a much higher number of third- than second-instar larvae in the samples despite the fact that second-instars were much more abundant in the field (fig. 2). This merely demonstrates that the wasps bring more of the larger instars back to their nests as food for their larvae. *Polistes* frequently kill larvae that are not carried to the nest. On July 27, 1954, 20 first-instar larvae and one large second-instar were placed on a plant and kept under close observation for 3 hours and 10 minutes. Eight larvae were killed by wasps. Four in the first instar were eaten on the spot, three more were killed or injured but not consumed. The second-instar larva and one of the injured firsts were carried away. Between July 30 and Aug. 7, 1956, hunting wasps in tobacco field C9a were followed. Fourteen individuals of 4 species were observed to kill 20 hornworms and budworms. Six of these were killed but not consumed, two were eaten on the spot, and 12 were formed into pellets and carried away. The average size of the hornworms killed and abandoned or eaten was 2.3 instars. Those formed into pellets averaged 3.4 instars.

The data in table 7 also indicate that *fuscatus* tends to prey on somewhat larger larvae than *exclamans*. Both species, however, were often observed to approach but leave large fifth-instar larvae unmolested.

Practical considerations.—The fact that *Polistes* feed almost entirely on free-feeding larvae limits their potentialities for the control of budworms, which feed within the tobacco bud during the period they are of most economic importance. These predators do, however, account for large reductions in hornworm populations and probably constitute a major mortality factor in the ecosystems of some other species of free-feeding larvae.

The number of hornworms lost from different causes during the first and second broods in tobacco (field C9a) are shown in table 8. Sixty-eight per cent of the hornworm larvae in both the first and second broods were killed by predators or disappeared. There is little doubt that most if not all of the missing larvae were carried off by diurnal predators (Lawson, unpublished). Many hours of observation in the field revealed that very few hornworms were killed by predators other than *Polistes*. On the other hand, *Polistes* were seen to kill many. Of 319 *Polistes* caught and marked in the tobacco field, there were 127 *fuscatus*, 121 *exclamans*, 56 *metricus*, 14 *annularis*, and 1 *rubiginosus*.

In spite of the heavy mortality, enough hornworms reached the fifth-instar in field C9a to cause economic damage by both broods. Damage inflicted by the second brood was particularly severe. The failure of the natural enemies to prevent hornworm damage may have been due to the abnormally high number of hornworm eggs laid (the number deposited in the second brood was the highest on record in N. C.) coupled with a relatively low population of natural enemies. The fact that field C9a was located in open crop land at least 600 feet from the nearest natural nesting site of *Polistes* and was surrounded by crops infested with larvae acceptable as prey by *Polistes* may also have had a depressing effect on predation in C9a.

Populations of prey other than hornworms are essential to the maintenance and growth of *Polistes* colonies, particularly before and between hornworm broods and in situations where hornworm populations are low. On the other hand, high populations of alternate prey at the time hornworm broods occur may have a marked effect in reducing the predation on hornworms. Therefore, it seems probable that *Polistes* are more effective in controlling hornworms under certain cropping systems than others. For example, predation might be expected to be greater in tobacco adjacent to corn and woods than in tobacco surrounded by soybeans or lespedeza, because populations of alternate prey are normally low on corn and fairly numerous on soybeans and lespedeza. Alternate prey in trees and woody shrubs might be of great importance when hornworm populations were low but would probably not seriously affect predations of *exclamans* and *fuscatus* in tobacco since these species seem to prefer hunting in herbaceous vegetation (Rabb 1953).

SUMMARY.—Investigations of several factors influencing the effectiveness of *Polistes exclamans exclamans* Vier. and *P. fuscatus fuscatus* (F.) in the natural control of the tobacco hornworm, *Protoparce sexta* (Johan.) were made near Clayton, North Carolina in 1956.

The seasonal variations in the abundance of hornworms

Table 7.—The instars of *Protoparce*, chiefly *sexta*, in prey samples taken from *Polistes* at the beginning and at the height of the second brood of third- and fourth-instar hornworms. Clayton, N. C., 1956.

Date of Collections	Species of *Polistes*	No. of *Protoparce* in Samples	Per Cent of Each Instar (2–5) in Samples			
			2	3	4	5
7-23-26	*exclamans*	50	26	64	10	0
	fuscatus	12	8	75	17	0
8-7-9	*exclamans*	86	10	56	28	6
	fuscatus	83	3	41	49	7

Table 8.—The number of hornworms per 25 plants lost from different causes during the first and second broods. Clayton, N. C., 1956.

Hornworm Brood	Eggs		No. Larval Instars 1 to 4 Killed by			No. Instar 5 Observed	Total Larvae	Total Eggs and Larvae
	Total	Lost	Found Dead[a]	Predators or Missing	Parasites[b]			
First	21	4	0	13	0	6	19	23[c]
Second	172	39	3	107	3	40[d]	153	192[e]

[a] Cause of death unknown.
[b] *Apanteles congregatus* Say.
[c] Two found as first-instar larvae but not observed as eggs.
[d] Losses in the fifth-instar are not shown, however, none died from parasitism before being killed by other causes or entering the ground.
[e] Twenty found as first-instar larvae but not observed as eggs.

and other free-feeding larvae were reflected in the samples of prey taken from wasps. In late June, a greater proportion of the prey of both species of *Polistes* was obtained from wooded areas than in July and August. The green cloverworm, *Plathypena scabra* (F.), although absent from prey samples in June, was one of the most numerous species taken by *Polistes* during July and August.

Within a distance of 800 feet from tobacco, the percentage of tobacco hornworms in the prey of *P. exclamans* was inversely proportional to the distance from tobacco. This was not clearly demonstrated for *fuscatus*, which is slightly larger and apparently flies a greater distance than *exclamans*. However, data on wasps marked in a field of tobacco and recovered on colonies at different distances from the point of marking, show an inverse relationship between distance and the number of wasps visiting the tobacco for *fuscatus* as well as *exclamans*.

That cultural practices may have a pronounced effect on the composition of *Polistes* prey was demonstrated when the percentage of green cloverworms in prey samples dropped sharply immediately after three large fields of soybeans were plowed under.

Most of the hornworms brought to their nests by *Polistes* wasps were third- and fourth-instars; however, the wasps were observed to kill many smaller larvae that they did not carry to their nests. In many cases, they also inspected but did not kill large fifth-instars.

P. e. exclamans and *P. f. fuscatus* have little effect in reducing damage from budworms, which feed within the protection of terminal buds during the period of their greatest economic importance. They do, however, bring about large reductions in populations of hornworm larvae. Predators, chiefly species of *Polistes*, killed 68% of the hornworm larvae in the experimental area, but, along with other natural enemies, failed to reduce the population below an economic level. This failure, however, is not considered representative because of the abnormally high hornworm population and the paucity of natural nesting sites for *Polistes* near tobacco. In addition, the abundance of alternate prey, particularly green cloverworms on soybeans, undoubtedly had a depressing effect on the number of hornworms killed by the wasps.

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The Biology and Control of *Eotetranychus multidigituli* (Ewing) a Spider Mite of Honey Locust[1]

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The development of desirable selections of the honey locust tree, *Gleditsia triacanthos* L., its presumed freedom from diseases and pests, and the decrease in popularity of other shade trees, notably the elm, have greatly increased the use of honey locust as a shade and ornamental tree. However, a two-year study of the life history and control of a spider mite, *Eotetranychus multidigituli* (Ewing), at Urbana, Illinois indicates that this mite can cause serious damage to the honey locust.

E. multidigituli, which will be referred to as the locust mite in this paper, pierces the leaf tissues with its mouthparts and sucks up the food substances. This feeding, which causes stippled and bleached spots of dead leaf tissue, for the most part takes place on the under sides of the leaflets. In case of severe infestations of the locust mite the feeding injury may be so great that the leaflets turn yellow and drop prematurely.

DISTRIBUTION.—The honey locust tree is the only

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known host for the locust mite. During the summer of 1956 the following five related species of trees were examined for mite infestations: the water locust, *Gleditsia aquatica* Marsh.; the black locust, *Robinia pseudoacacia* L.; the redbud, *Cercis canadensis* L.; the yellow-wood, *Cladrastis lueta* (Michx. f.) K. Koch; and the Kentucky coffee-tree, *Gymnoclodus dioica* (L.) K. Koch. Although these trees were infested with the two-spotted spider mite, *Tetranychus telarius* (L.), none of the trees examined was infested with the locust mite.

The locust mite has been collected from the following places: Wooster, Ohio (Ewing 1917); New Haven and Glastonbury, Connecticut (Garman 1940); Hamden, Connecticut, Washington, D.C., Durham, North Carolina, Natchitoches, Louisiana (Pritchard & Baker 1955); and from Belleville, Carbondale, Champaign, Chicago, Dwight, Forrest, Lake Zurich, Mattoon, Morris, Niles, Park Forest, Sibley, Strawn, Urbana, Vandalia and Yorkville, Illinois by the authors.

Eotetranychus multidigituli (Acarina: Tetranychidae) was first described by Ewing (1917) under the name of *Tetranychus multidigituli*. McGregor (1920) reviewed Ewing's description. Garman (1940) described a mite from honey locust and named it *Tetranychus ellipiticus*.

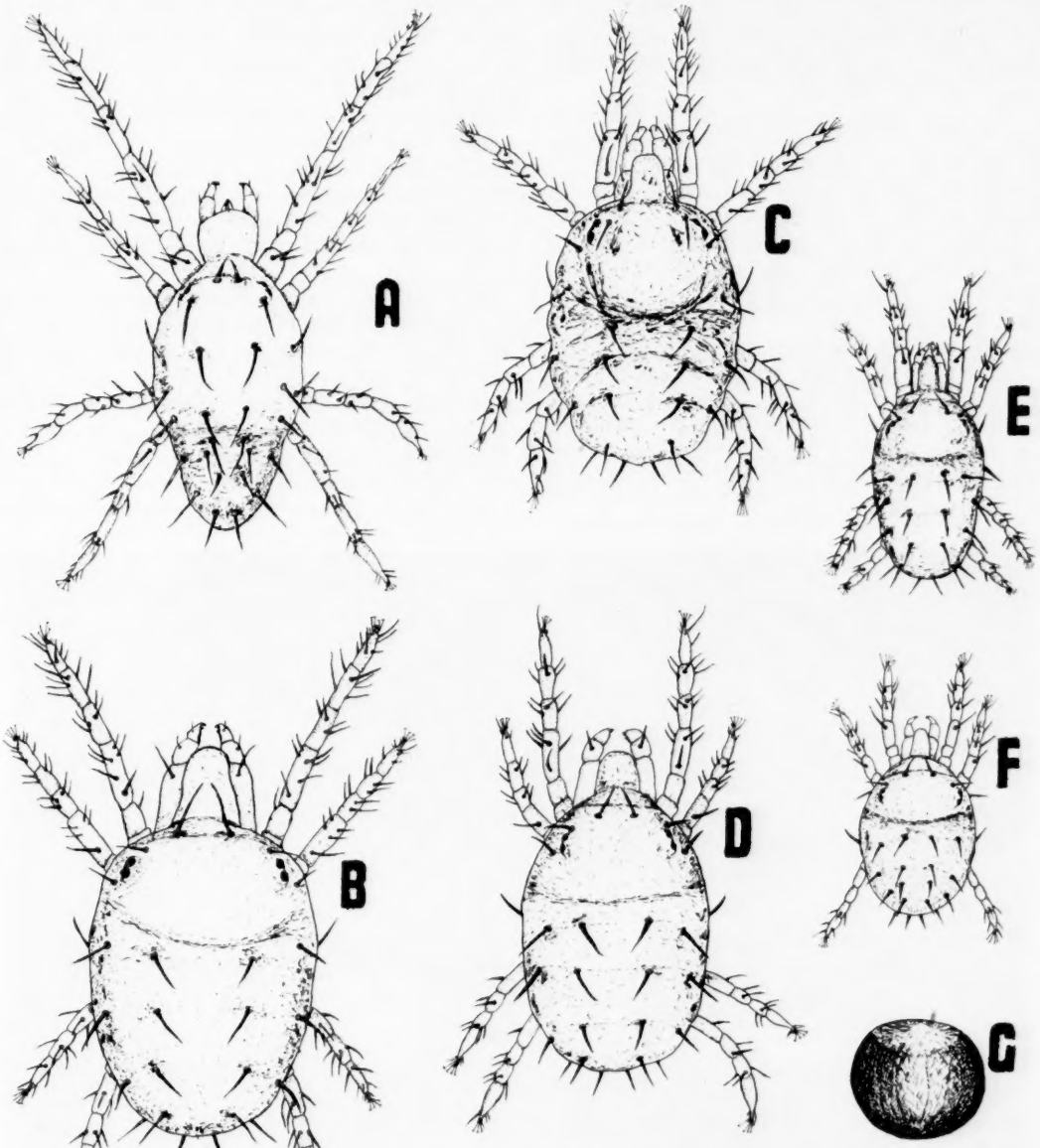

FIG. 1.—The life stages of the locust mite, *Eotetranychus multidigituli* (Ewing): (A) Dorsal view of adult male; (B) Dorsal view of of adult female (summer form); (C) Dorsal view of adult female (winter form); (D) Dorsal view of deutonymph; (E) Dorsal view of protonymph; (F) Dorsal view of larva; (G) Side view of egg.

McGregor (1950) in his work on the spider mites reviewed both descriptions. In their revision of the family Tetranychidae, Pritchard & Baker (1955) recognized *T. ellipiticus* as a synonym of *T. multidigituli*. In addition they assigned the mite to the genus *Eotetranychus* Oudemans.

MORPHOLOGY.—The life stages of the locust mite are: the egg, the larva, the protonymph, the deutonymph, and the adult (male and female). The larval, protonymphal and the deutonymphal stages have both active and resting phases. The adult female has a summer form and an overwintering form.

Egg.—The egg is minutely striated above, without a central papilla, circular in dorsal view, slightly depressed and flattened in lateral view, fig. 1 (G). The color is pearly with a slight yellowish tinge. The average dorsal measurement of the egg is 0.114 mm. and the average lateral measurement is 0.097 mm.

Larva.—The body of the six-legged larva is elliptical in dorsal view, fig. 1 (F). The larva is like the succeeding stages in that it has two pairs of eyes which are conspicuous because of their red color. As the larva feeds, its body color changes from a pale yellow to dark green. The average length of the newly hatched larva is 0.138 mm. and the average width is 0.091 mm. The average length of the larva in the resting phase is 0.161 mm. The average width is 0.124 mm.

Protonymph.—The protonymph has eight legs and is larger than the larva, fig. 1 (E). The body color is greenish-yellow to dark green with vague spots along the sides. The average body length of the active phase is 0.225 mm. and the average width is 0.165 mm. The average body length of the resting phase is 0.222 mm. and the average body width is 0.161 mm.

Deutonymph.—The deutonymph is considerably larger than the protonymph, fig. 1 (D). The body color of the deutonymph is similar to that of the protonymph. The average body length of the active phase is 0.282 mm. and the average body width is 0.182 mm. The average body length of the resting phase is 0.286 mm. and the average body width is 0.195 mm.

Adult Male.—The male is smaller than the female and his abdomen is much narrower at the rear than that of the female, fig. 1 (A). The legs, particularly the first pair, of the male are much longer than those of the female. The average body length of the male is 0.259 mm. and the average body width is 0.161 mm.

Adult Female.—The summer form of the female is nearly elliptical in shape, but is slightly narrowed at the rear, fig. 1 (B). The body length of the summer female is is 0.332 mm. and the average width is 0.222 mm. The winter form of the female, fig. 1 (C), is considerably smaller than the summer female. The legs are much shorter. Instead of the light green to dark green color of the male and the summer female, the winter female has an orange-red color. The average body length of the winter form of the female is 0.211 mm. and the average body width is 0.141 mm.

REARING TECHNIQUE.—Holes about $\frac{1}{4}$ inch in diameter were cut in the lids of plastic refrigerator boxes about $3\frac{1}{2}\times4\frac{1}{2}\times2$ inches in size. A piece of glass tube, about $2\frac{1}{2}$ inches long and fire polished on both ends, was placed in each hole. Each mite was placed on a cropped honey locust leaflet, where it was confined by a small ring of Tree Tanglefoot on the petiole a short distance from the leaflet. The petiole of each leaflet was inserted into one of the glass tubes in the plastic box containing distilled water.

FIG. 2.—Equipment used for rearing individual mites.

For convenience, two of the plastic boxes were kept in a larger plastic box, of the type commonly known as a bread box, fig. 2. The lid of the bread box was used to cover the cultures while they were being carried between the laboratory and an open shelter out of doors.

Daily observations on individual mites began in the latter part of May and continued through September. A culture was started by transferring an adult female from natural habitat to a leaflet with the aid of a bristle glued to a piece of cane. The female was transferred to a fresh leaflet each day eggs were laid. The leaflet with a day's egg deposit usually remained fresh throughout the incubation period. Larvae were transferred to fresh leaflets daily and successive active stages were transferred when necessary.

DEVELOPMENT.—Male and female locust mites have the same number of stages and molts during development from egg to adult. One hundred individuals were observed through complete development. The incubation period, which varied from 4 to 9 days, depended on the weather. In warm weather the eggs hatched usually in 5 days, in cooler weather in 7 or 8 days. The average incubation period was 5.8 days.

The time required for development from larva to adult ranged from 4 to 11 days and averaged 4.9 in June and 8.5 days in September. Total development (oviposition to adult) required from 8 to 17 days, and averaged 9.5 days in June and 14.6 days in September. The incubation period was roughly about half of the time required for total development. The pre-oviposition period was usually less than 1 day, sometimes 1 day, and rarely 2 days.

It was typical for a mite to spend 1 day in each active phase and each resting phase. In no case did the larva spend less than 1 day in the active phase. A few larvae required 2 days and one required 3 days. In the two nymphal stages both the active and resting phases sometimes required less than 1 day. However, in cool weather 2 or 3 days were required before a change was made.

The male developed a little faster than the female. The average for total development for 45 males was 12.0 days and for 55 females 12.7 days. This is an unimportant difference, as several brothers and sisters from eggs laid the same day completed development simultaneously.

REPRODUCTION.—Male mites wait beside female deutonymphs during the last resting period. Shortly after the females molt copulation takes place.

Of 847 eggs observed, 468 or 55.3% were deposited on the under sides of the leaflets, 17.2% on the upper sides, and 27.5% on the leaflet petioles. Only 2.5% of the eggs failed to hatch. The females laid an average of 5.2 eggs per day. The highest number of eggs laid in 1 day by an individual was 11. One female, observed for 11 days, laid a total of 72 eggs.

Of 83 mites reared from females presumed to be fertile, 55 were females and 28 were males. The 17 individuals reared from virgin females were all males. Hence, *E. multidigituli* may reproduce bisexually or parthenogenetically.

HIBERATION.—With the approach of cool weather in the fall, female mites move to the twigs and branches, gradually become orange red in color and almost inactive. In this condition they pass the winter under the rough bark at the base of twigs and under bud scales. Some females were seen hibernating under the shells of dead Putnam scale, *Aspidiotus ancylus* (Putn.). No overwintering males were observed.

The overwintering females die in the spring after laying eggs on the twigs. In 1956 eggs were first observed on April 11, and a few larvae were observed on May 1. As new leaves develop, the mites move to them to feed.

SUMMER ACTIVITY.—Estimates of the mite population during the summer of 1956 were made as follows: 32 compound honey locust leaves were collected at weekly intervals from each of three trees in different locations. The leaf samples were placed in plastic bags and taken to the laboratory, where the mites on each leaf were brushed onto a glass plate with a machine designed by Henderson & McBurnie (1943). The number of mites was counted on a $\frac{1}{32}$-sector of the glass plate to give the per-leaf estimate of the population.

Only light infestations developed on trees 1 and 2, whereas the infestation on tree 3 became very heavy in July and August, fig. 3. The infestation on tree 1 was very light until after the middle of July, but continued later in the season than the infestations on trees 2 and 3. The infestation on tree 2 reached a peak of 23 mites per leaf on July 30 and dropped to inconsequential numbers by the middle of August. The infestation on tree 3 rose rapidly from 7 mites per leaf on June 18 to 176 mites per leaf on July 16. The infestation remained high for 4 weeks and then dropped sharply to only 5 mites per leaf September 4. Much of the foliage on tree 3 turned yellow and dropped prematurely.

NATURAL ENEMIES.—The following predators were observed feeding on the locust mite: *Stethorus punctum* Lec., *Scolothrips sexmaculatus* (Perg.) and *Typhlodromus fallacis*

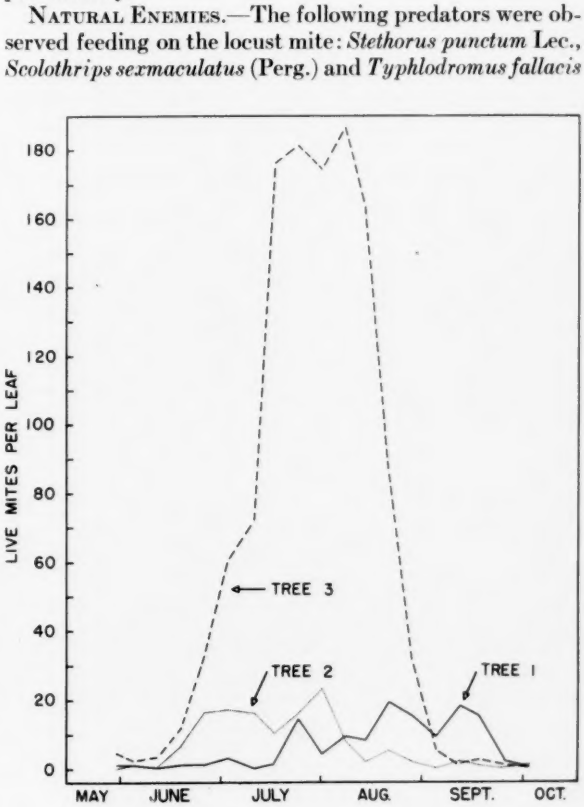

FIG. 3.—Abundance of the locust mite on three honey locust trees during the summer of 1956.

Table 1.—The effectiveness of sprays against the locust mite, applied July 7, 1955.

MATERIAL PER 100 GAL. (POUNDS)	NO. OF LIVE MITES PER LEAF ON DATES INDICATED								
	7/6	7/11	7/19	7/26	8/3	8/10	8/17	8/31	9/14
Malathion 25-W, 1	58	1	1	3	6	1	1	0	3
Aramite 15-W, 1	58	11	1	2	4	2	3	1	1
Malathion, 1 & Aramite, 1	50	2	0	1	1	3	4	0	1
Control	13	12	3	27	49	11	3	1	3

(Garman). Of these *T. fallacis* was the most numerous. Just how important this predator may be in the natural control of the locust mite is not known. Light infestations on some trees indicate the activity of some agency, but heavy infestations on other trees indicate the ineffectiveness of natural agencies. Weekly records from one such tree showed that *T. fallacis* did not become abundant until the latter part July. Previous to this time a heavy infestation of the locust mite had developed.

CONTROL WITH SPRAYS.—Population estimates by the leaf brushing technique, described above, were used to evaluate the effectiveness of several sprays. In 1955, plots consisting of two trees each, were sprayed with a power sprayer on July 7 using malathion 25-W, Aramite 15-W, and a combination of the two. All three sprays substantially reduced the mite population and maintained a practical level of control the remainder of the season (table 1).

In 1956, plots containing four trees were treated with sprays containing malathion emulsion, malathion wettable powder, and combinations of malathion with Aramite and chlorobenzilate. All four sprays seemed to eliminate the infestations (table 2).

SUMMARY.—Observations on the biology and control of the spider mite, *Eotetranychus multidigituli* (Ewing), a pest of honey locust, *Gleditsia triacanthos* L., were made over a 2-year period. The mite feeds mainly on the under sides of the leaflets and causes them to turn yellow and drop prematurely. This mite is yellowish-green, lays eggs, and has a larval stage, two nymphal stages, with corresponding resting periods, and an adult stage. The female overwinters as an orange-red, inactive form and deposits eggs on the twigs first and later on the leaflets. The summer population reaches a peak in July and August.

Individual mites were reared on cropped honey locust leaflets in a field shelter during the summer of 1956. The eggs hatched in 5 to 7 days and development from larva to adult took place in 4 to 11 days. The mite commonly spent 1 day in each active phase and each resting phase. Development from egg to adult required an average of 9.5 days in June and 14.6 days in September. Females laid an average of 5.2 eggs per day, of which 97.5% hatched. *E. multidigituli* reproduces both bisexually and parthenogenetically. All the progeny of virgin females were males.

Typhlodromus fallacis (Garman) was the most common of several predators observed. However, natural enemies failed to prevent heavy infestations of *E. multidigituli* on many trees. Aramite, chlorobenzilate, and malathion were effective sprays.

Table 2.—The effectiveness of sprays against the locust mite, applied August 6, 1956.

TREATMENT	NO. OF LIVE MITES PER LEAF ON DATES INDICATED			
	7/31	8/14	8/27	9/10
Malathion[a]	95	0	0	0
Malathion[b]	81	0	0	0
Malathion & Aramite[c]	101	0	0	0
Mal. & Chlorobenzilate[d]	142	0	0	0
Control	118	34	9	3

[a] 1 qt. 57% emulsion/100 gal.
[b] 4 lbs. 25-W/100 gal.
[c] 4 lbs. 25-W plus 1 lb. Aramite 15-W/100 gal.
[d] 4 lbs. 25-W plus 1 lb. chlorobenzilate 25-W/100 gal.

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Organic Phosphorus Insecticides as Residual Sprays for the Control of House Flies[1]

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Organic phosphorus insecticides have been tested extensively as residual sprays in areas where house flies (*Musca domestica* L.) have become resistant to the chlorinated hydrocarbons. At the present time Diazinon appears to be one of the outstanding materials. Kocher *et al.* (1953) and Gasser (1953) reported that it was used successfully during 1951–52 in houses and animal quarters in the Valais region of Switzerland. The residues from 0.1 to 0.125% Diazinon suspensions were effective for 4 to 10 weeks in places where the insects had become resistant to DDT, chlordane, BHC, and dieldrin. The first reported use of Diazinon residual sprays in the United States was by Hansens & Bartley (1953) in New Jersey, where they obtained excellent control of flies for the 1952 season in a horse barn and for 3 to 4 weeks in dairy barns. Hansens & Scott (1955) found residues of this insecticide remained effective for 40 to 50 days.

Other phosphorus compounds have been tried also. In California dairy barns Hoffman & Cohen (1954) found Bayer 21/199, Dipterex (Bayer L 13/59), Chlorthion, malathion, and propyl thiopyrophosphate (NPD) were not promising at 50 and 100 mg. per square foot, but Diazinon was more than 90% effective for a short period. Kilpatrick & Schoof (1954) reported that malathion and propyl thiopyrophosphate at 100 to 200 mg. per square foot maintained good control for 1 to 3 weeks in dairies near Savannah, Ga., that were infested with resistant flies. Dahm & Raun (1955) obtained control with Am. Cyanamid 4124, Chlorthion, Diazinon, malathion with butoxy polypropylene glycol (Penthion 80), and Pirazinon on Iowa farms, and Hansens & Scott (1955) found Pirazinon nearly equal to Diazinon in New Jersey. In other tests Hansens *et al.* (1955) and Hansens (1956) reported that Am. Cyanamid 4124 gave excellent control for 6 to 8 weeks and was better than Chlorthion, Dow ET-15, methoxychlor, malathion, and lindane, but inferior to Diazinon and Dow ET-57 (sampled as Dow ET-14). Diazinon also was superior to malathion in Maryland (Johnson *et al.* 1956) and Wyoming (De Foliart 1956). Am. Cyanamid 4124 was equal but not superior to malathion in the tests in Maryland.

DDT residues failed to give adequate control of house flies in the animal barns of central Florida as early as 1948 (King & Gahan 1949). By the fall of 1950 chlordane, lindane, and dieldrin also were unsatisfactory, and it became obvious that an entirely different type of chemical would be needed to reestablish good control.

Thirty of the organic phosphorus compounds that were found highly toxic to mosquito larvae or body lice at the Orlando, Fla., laboratory have been evaluated in the laboratory as residual sprays against adult house flies. Seven of those that showed some toxicity and were commercially available were tested further in animal barns in central Florida. Diazinon and malathion were used on Nebraska farms. All the buildings were heavily infested with adult flies before the treatments were applied. This paper summarizes the results that have been obtained with these materials between 1952 and 1956.

Laboratory Tests.—The insecticides were applied in acetone to plywood panels 1 foot square, at the rate of 100 mg. of toxicant per square foot. Ten milliliters of a 1% solution was used on each board. Twenty female house flies from a strain nonresistant to the chlorinated hydrocarbons were confined under a half section of a petri dish on the treated surfaces. After exposures of 5, 15, 30, 60, or 120 minutes the insects were transferred to screen holding cages, furnished honey and water, and the mortality in 24 hours was recorded. Duplicate tests were run the day after treatment and at gradually increasing intervals until it became obvious the residues were quite durable or not highly effective.

The nine phosphorus compounds listed in table 1 with the DDT standard were sufficiently toxic that a 120-minute exposure caused 98 to 100% mortality for at least 14 days. The most promising materials were Dow ET-15, Dow ET-57, Diazinon, Bayer 21/199, and EPN. The first two materials were the only ones that consistently caused 98 to 100% mortalities throughout the first 28 days, after exposures as short as 5 to 15 minutes. A sufficient number of exposures were not made to clarify the position of EPN in relationship to these four insecticides, but it was definitely superior to the remaining compounds. Methyl parathion and para-oxon also killed all the flies at one or more exposure periods on the 28th day, but 2-chlorovinyl dimethyl phosphate and dibutyl 2,2-dichlorovinyl phosphate lost most of their effectiveness after the 14th day. All the compounds except dibutyl 2,2-dichlorovinyl phosphate were superior to DDT in the 5- and 15-minute exposure tests run on freshly treated boards, but only Dow ET-15 and Diazinon were equal to this standard by the 56th day.

The following compounds failed to cause 98% mortality on the 14th day after treatment:

Acetate of dimethyl 2,2-dichloro-1-hydroxyethylphosphonate
Acetate of dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate
Am. Cyanamid 4124
Bayer 21/200
Bayer 28/63
S-2-(Chloroethyl) *O,O*-diethyl phosphorothioate
2-Chlorovinyl dipropyl phosphate
Demeton
O,O-Diethyl *O*-(2-diethylaminoethyl)phosphorothioate
Dimethyl 1-butyryloxy-2,2,2-trichloroethylphosphonate
Dimethyl 1,2,2,2-tetrachloroethyl phosphate
Dipropyl 1,2,2,2-tetrachloroethyl phosphate
Dipterex
Guthion
Malathion
Octanoate of dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate
Potasan
Sulfotepp
TEPP

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Table 1.—Toxicity of nine phosphorus compounds and a DDT standard to female house flies in laboratory tests.

Chemical	Age of Residue (Days)	Per Cent Kill in 24 Hours After Exposures of—				
		5 Min.	15 Min.	30 Min.	60 Min.	120 Min.
Dow ET-15	1	100	100	100	100	100
	7	85	100	100	100	100
	14	100	100	100	100	100
	28	100	100	100	100	100
	56	20	55	100	100	100
	180	—	—	98	100	100
Dow ET-57	1	100	100	100	100	100
	7	100	100	100	100	100
	14	98	100	100	100	100
	28	98	100	100	100	100
	56	23	3	45	73	100
	180	—	—	—	—	100
Diazinon	1	88	100	100	100	100
	7	85	100	100	100	100
	14	30	98	100	100	100
	28	40	78	100	100	100
	56	28	65	90	98	100
Bayer 21/199	1	83	100	98	100	100
	7	88	95	100	100	100
	14	83	100	90	100	100
	21	63	100	100	100	100
	28	3	13	98	100	100
	56	3	30	55	100	100
EPN	1	—	100	100	100	100
	7	—	43	100	100	100
	28	—	98	100	100	100
Methyl parathion	1	—	100	100	100	100
	7	—	95	100	100	100
	28	—	15	53	80	100
Para-oxon	7	—	95	98	100	100
	28	—	8	48	95	100
2-Chlorovinyl dimethyl phosphate	1	100	100	100	100	100
	7	25	75	100	100	100
	14	0	3	5	53	100
	28	—	—	—	40	50
Dibutyl 2,2-dichlorovinyl phosphate	1	43	75	100	100	100
	7	43	68	100	100	100
	14	3	0	18	28	95
	28	—	—	—	—	20
DDT	1	20	35	93	85	100
	7	43	70	88	83	95
	14	23	65	90	98	98
	28	83	100	95	100	100
	56	18	60	98	100	100
	180	—	—	98	100	100

TESTS IN BUILDINGS.—Sprays containing Diazinon, Dow ET-57, Dipterex, Chlorthion, Am. Cyanamid 4124, Bayer 21/199, and malathion were applied in a number of buildings in Florida where the strains of flies present were known to be moderately to highly resistant to all the chlorinated insecticides. Diazinon, malathion, and DDT were used in barns on Nebraska farms where residual sprays had not been used as extensively, and the resistance of the flies to insecticides was not known. Dipterex was used in a water solution. Malathion, Chlorthion, and DDT were used in emulsions, and the other compounds in emulsions and wettable-powder suspensions.

The insecticides were applied at 25 to 200 mg. per square foot with a power sprayer equipped with *Tee-Jet* 80-02 nozzles, which produce a flat spray. The sprayer was operated at 75 p.s.i. pressure. One gallon of spray was used per 1,000 square feet of surface. Most of the treatments were applied to the walls and ceilings, but in one test with Bayer 21/199 wettable powder the floor was sprayed also. In other tests the Bayer 21/199 was used only on the floor at 200 mg. per square foot, and enough Diazinon to treat the walls and ceiling at 100 and 200 mg. per square foot was applied to the ceiling only. Sugar was included at 300 to 450 mg. per square foot in some of the formulations containing Diazinon, Chlorthion, Am. Cyanamid 4124, Bayer 21/199, and malathion.

The effectiveness of the treatments was determined by comparing the numbers of flies present before and at various intervals after application. The population density was based on counts of 1- by 24-inch strips of cloth that had been dipped in a 50% solution of malt in water. Counts were made at five locations in the barns where flies were most abundant. Results of these tests are presented in table 2.

In Florida barns Diazinon gave 90 to 100% control for periods ranging from 3 days to at least 7 weeks. Although some of the deposits at 25 and 50 mg. per square foot outlasted some of those at 100 mg., 100 mg. usually produced the best results. Treatments applied to both the walls and ceilings were superior to those applied only to the ceilings, even when the total rate per barn was the same. Dow ET-57 remained highly effective from 1 to 28 days, and applications at 50 mg. were about as durable as those at 100 and 200 mg. per square foot. Am. Cyanamid 4124 gave more than 90% control for 1 to 14 days. Malathion, Dipterex, Bayer 21/199, and Chlorthion usually gave 90% control for no more than 1 week, but one 200-mg. deposit of Dipterex lasted about 14 days. Treating the floor as well as the walls and ceiling did not improve the results obtained with Bayer 21/199. This insecticide, with and without 10% sugar in the formulation, was unsatisfactory when only the floor of the feed room was sprayed at 200 mg. per square foot. Inclusion of sugar in the spray formulation did not greatly extend the durability of any of the residues, and caused unsightly black mold on many of the barns.

Under conditions prevailing in Nebraska animal barns and shelters, Diazinon was more effective than malathion or DDT as a wall and ceiling residual treatment. It gave satisfactory control for 9 to 35 days at 50 to 100 mg. per square foot, whereas malathion at 100 and 200 mg. was satisfactory for 1 week or less. DDT was the poorest of the three. Both Diazinon and malathion were more effective in Nebraska than in Florida. Two weeks after treatment the only flies resting at night or very early in the morning in the buildings sprayed with Diazinon were on remote areas that had been difficult to spray or that were covered with dust, spider webs, or fly feces. Many affected flies were found on the floor. Knocked-down flies were collected from the floor 2 weeks after treatment of the barn, and less than 25% of them recovered within 24 hours. Two weeks later the recovery rate from other collections ranged between zero and 56%.

Table 2.—Control of natural populations of house flies in animal barns.

TOXICANT AND FORMULATION	TOXICANT PER SQUARE FOOT (MG.)	PER CENT CONTROL AT INDICATED DAYS AFTER TREATMENT										
		1	3–4	7	9–11	14	21	28	35	42	49	56
		Florida tests										
Diazinon:												
Emulsion	25	99	99	99	98	99	86	91	90	79	—	—
		99	99	93	96	97	92	77	74	—	—	—
	50	99	99	46	0	—	—	—	—	—	—	—
		96	96	42	25	31	—	—	—	—	—	—
		99	99	99	99	92	90	80	95	97	85	82
		99	97	92	99	92	84	94	86	66	—	—
		99	99	85	94	65	27	14	0	—	—	—
		99	99	92	97	93	0	—	—	—	—	—
	100	99	99	99	99	70	76	70	68	—	—	—
		99	98	98	99	99	99	98	91	68	94	70
		100	99	99	96	95	95	96	0	—	—	—
		98	98	99	91	97	97	96	97	76	90	86
		99	98	99	98	98	98	98	95	90	93	—
		98	99	96	98	99	97	93	97	97	97	—
		98	96	91	95	97	99	73	0	—	—	—
	100[a]	96	95	0	—	—	—	—	—	—	—	—
	100[b]	99	99	99	96	99	97	96	92	98	—	—
		99	99	94	96	94	94	87	—	—	—	—
	200[c]	99	99	99	93	86	72	0	—	—	—	—
Suspension	25	0	—	—	—	—	—	—	—	—	—	—
		0	—	—	—	—	—	—	—	—	—	—
Dow ET 57:												
Emulsion	50	86	97	93	97	88	31	—	—	—	—	—
	100	99	97	94	91	98	84	0	—	—	—	—
		99	96	90	90	73	31	—	—	—	—	—
		99	96	92	86	96	95	96	72	83	45	—
	200	84	64	92	89	93	75	48	—	—	—	—
Suspension	50	98	95	97	96	95	95	93	65	67	0	—
		72	90	79	90	88	0	—	—	—	—	—
	100	92	80	70	89	84	50	—	—	—	—	—
		99	96	90	87	86	57	—	—	—	—	—
Am. Cyanamid 4124:												
Emulsion	100[b]	97	84	79	—	—	—	—	—	—	—	—
		98	95	91	96	86	79	88	77	—	—	—
Suspension	100[b]	99	99	98	93	85	86	72	—	—	—	—
		99	99	94	72	84	—	—	—	—	—	—
Dipterex solution	200	99	99	92	92	91	0	—	—	—	—	—
		99	98	66	0	—	—	—	—	—	—	—
Bayer 21/199:												
Emulsion	50	88	83	73	—	—	—	—	—	—	—	—
	100[b]	70	84	6	—	—	—	—	—	—	—	—
Suspension	100	82	97	88	0	—	—	—	—	—	—	—
		96	96	93	0	—	—	—	—	—	—	—
		98	97	91	65	—	—	—	—	—	—	—
	200	73	44	—	—	—	—	—	—	—	—	—
	200[d]	91	94	76	0	—	—	—	—	—	—	—
	200[b,e]	72	0	—	—	—	—	—	—	—	—	—
	200[e]	82	92	0	—	—	—	—	—	—	—	—
Malathion emulsion	25	3	48	—	—	—	—	—	—	—	—	—
	25[b]	93	69	63	78	80	57	—	—	—	—	—
		78	98	92	76	48	—	—	—	—	—	—

(Continued on next page)

Table 2.—(*continued*)

Toxicant and Formulation	Toxicant per Square Foot (Mg.)	Per Cent Control at Indicated Days After Treatment										
		1	3–4	7	9–11	14	21	28	35	42	49	56
Malathion emulsion (*continued*)	50	93	93	93	76	50	—	—	—	—	—	—
	100	93	50	—	—	—	—	—	—	—	—	—
	100[b]	99	98	89	58	23	—	—	—	—	—	—
		93	57	41	—	—	—	—	—	—	—	—
	200	87	94	42	—	—	—	—	—	—	—	—
		92	70	86	63	—	—	—	—	—	—	—
		97	97	32	24	0	—	—	—	—	—	—
Chlorthion emulsion	100[b]	94	80	51	—	—	—	—	—	—	—	—
		94	91	75	86	86	81	77	—	—	—	—
		Nebraska tests										
Diazinon emulsion	50	99	97	98	99	81	76	64	74	10	—	—
		99	95	94	98	95	77	92	77	50	—	—
		96	84	96	95	94	97	98	86	17	—	—
		97	95	94	96	97	99	96	96	69	—	—
	100	99	90	89	94	90	80	68	64	30	—	—
		99	96	98	99	98	96	98	—	40	—	—
		99	95	96	98	97	96	95	30	30	—	—
		99	97	96	97	94	96	93	48	—	—	—
Malathion emulsion	100	99	92	58	63	17	39	28	—	—	—	—
		99	96	76	62	0	16	6	—	—	—	—
		95	78	89	73	39	29	33	—	—	—	—
		71	83	67	65	—	—	—	—	—	—	—
		99	96	90	81	71	68	23	—	—	—	—
	200	99	97	96	89	68	71	10	—	—	—	—
		85	89	93	52	0	0	0	—	—	—	—
		78	92	89	87	71	53	—	—	—	—	—
		25	88	82	28	0	0	—	—	—	—	—
		90	91	90	29	0	0	—	—	—	—	—
DDT emulsion	200	89	77	97	13	25	—	—	—	—	—	—
		61	54	32	40	30	—	—	—	—	—	—

[a] Enough insecticide was used to treat the walls and ceiling at 100 mg. per square foot, but it was all applied to the ceiling.
[b] 300 to 450 mg. of sugar per square foot.
[c] Enough insecticide was used to treat the walls and ceiling at 200 mg. per square foot, but it was all applied to the ceiling.
[d] 1,200 square feet of floor treated in addition to walls and ceiling.
[e] Only floor of feed room treated.

In both Florida and Nebraska the actual length of control varied with conditions on the various premises, and in Florida seasonal differences also occurred. In general, residual control was of shorter duration in Florida than that reported from other sections of the country.

SUMMARY.—Of 30 materials evaluated in the laboratory as residual sprays against house flies (*Musca domestica* L.), the most promising were Dow ET-15, Dow ET-57 (sampled as Dow ET-14), Diazinon, Bayer 21/199, and EPN.

In Florida barns Diazinon residues at 50 to 200 mg. per square foot gave 90 to 100% control for periods ranging from 3 days to at least 7 weeks Treatments applied to both the walls and ceilings were superior to those applied only to ceilings. Dow ET-57 remained highly effective for 1 to 28 days and Am. Cyanamid 4124 was satisfactory for 1 to 14 days. These three chemicals were better than malathion, Dipterex, Bayer 21/199, and Chlorthion, which usually lasted no more than 1 week.

Diazinon and malathion were both slightly more effective under conditions prevailing in Nebraska than in Florida. Diazinon gave satisfactory control for 9 to 35 days at 50 to 100 mg. per square foot, whereas malathion at 100 and 200 mg. was satisfactory for 1 week or less. DDT was poorer than either of these materials.

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Greenbug Populations and Their Damage to Winter Wheat as Affected by Fertilizer Applications[1]

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Relationships between plant nutrients and insect infestation have been discussed in several papers. Painter (1951) stated that "each species of insect, in relation to its host plant, may be affected by soil conditions in respect to one or more factors of resistance." Haseman (1946) reported a higher rate of reproduction of chinch bugs, *Blissus leucopterus* (Say), when nitrogen was withheld from the nutrient solution in which corn plants were grown as food. There is little specific work on the effects of plant nutrients on the greenbug, *Toxoptera graminum* (Rond.). However, observations support the theory that small grains grown in soils with nutrients optimum for healthy, vigorous plants are more capable of withstanding greenbug infestations. Dahms (1951) recommended cultural measures that would stimulate the growth of small grains and enable them to better withstand greenbug attack. Blickenstaff *et al.* (1954) reported that in oats and rye greenbug populations decreased as the rate of nitrogen was increased. Investigations by Arant & Jones (1951) showed greenbug populations varied inversely with the amount of nitrogen applied to oats.

Greenbug infestations are common in the High Plains of Texas and fertilizer practices vary. Therefore, greenhouse experiments were conducted at the Southwestern Great Plains Field Station at Bushland, Texas, to determine certain effects of fertilizer combinations on greenbug populations and their damage to winter wheat. Data were also obtained from a fertilizer experiment at Farwell Texas, 90 miles southwest of Bushland, when a greenbug infestation developed on the plots.

MATERIALS AND METHODS.—*Greenhouse Experiment.*—Eight-inch pots were filled with Pullman silty clay loam soil obtained from land on which wheat had been grown. Fertilizers were applied in a band 1 inch below and 0.5 inch to the side of the seed row. Westar wheat was planted and later thinned to four plants per pot. The treatments listed in table 1 were arranged in a split plot design and replicated four times. Whole plots consisted of two pots of each fertilizer treatment. Each whole plot was divided into subplots of one pot. One subplot was infested with greenbugs and the other left as a noninfested check. This test was conducted in 1956 and again in 1957.

One-half gallon Mason jars, with the bottoms removed, were used as plant cages. The jars were capped with 70-mesh brass screen. Noninfested as well as infested plants were caged to provide similar growing conditions. The wheat plants were each infested, when about 3 inches tall, with two adult greenbugs.

Population counts were taken 30 days after infestation. Counts were made by clipping, at random, half the plants of each infested pot and brushing the greenbugs from the plants onto a white gridded cardboard where they were counted. Green weights were taken for comparison at the time of count. The number of greenbugs per gram of foliage was calculated.

Nitrogen was supplied as ammonium sulfate (21% N), phosphorus treble superphosphate (45% P_2O_5), potassium as muriate of potash (60% K_2O), and calcium as gypsum (74% $CaSO_4$). Local reports indicated that the addition of calcium to the soil reduced greenbug damage, therefore, it was included in this experiment.

Field Experiment.—Fertilizer treatments, table 3, were applied to Amarillo fine sandy loam soil in irrigated plots 10×50 feet in size and replicated three times. Nutrients were supplied by ammonium sulfate (21% N) and treble superphosphate (45% P_2O_5). Fertilizer applications were made, 1 day prior to seeding, with a multicell belt type applicator. The nutrients were applied 6 inches deep in bands 1 foot apart. Comanche wheat was seeded September 13, at the rate of 63 pounds per acre.

Greenbug counts were taken, April 1, by counting the insects on 1 linear foot of drill row at three random loca-

Table 1.—Mean number of greenbugs on two wheat plants and mean number of greenbugs per gram of foliage of infested plants.

Treatment, Pounds per Acre				Populations			Greenbugs per Gram of Foliage		
N	P_2O_5	K_2O	$CaSO_4$	1956	1957	Mean	1956	1957	Mean
0	0	0	0	199	501	350	470	724	597
0	60	0	0	177	331	254	507	781	644
60	0	0	0	183	332	257	327	509	418
60	60	0	0	180	313	246	316	338	327
60	60	60	0	193	366	276	339	393	366
60	0	0	1000	249	537	393	608	850	729

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tions within each plot. Foliage clippings, two 1-foot samples per plot, were taken and the number of greenbugs per gram of foliage calculated.

RESULTS.—*Greenhouse Experiment.*—The mean number of greenbugs per two wheat plants and the mean number of greenbugs per gram of foliage are presented in table 1. Analysis of variance of these data showed that greenbugs per gram of infested foliage differed significantly where nitrogen was applied. Less insects per gram occurred when nitrogen was used without calcium sulfate. The use of phosphorus and potash apparently made little difference. When phosphorus was used alone greenbugs per gram of foliage were as numerous as when no nutrients were applied. With the nitrogen-calcium sulfate combination both greenbugs per plant and greenbugs per gram of foliage were higher than on the other treatments.

Infested plants of all treatments were damaged by greenbugs, table 2. However, less damage occurred when nitrogen was applied, with the exception of the nitrogen-calcium sulfate combination. The plants on this treatment were more severely damaged than on the other treatments where nitrogen was included. Analysis of variance showed that no significant differences in weight occurred among treatments of the noninfested plants. However, there was a significant difference in weights among treatments of the infested plants. The treatments that received nitrogen, with the exception of the nitrogen-calcium sulfate treatment, had higher mean weights than those without nitrogen. This is indicative that nitrogen or some nutrient combination may reduce greenbug damage. However, under conditions of a previous greenhouse test conducted by Daniels & Porter (1956), less damage observed for plants grown on higher nitrogen levels appeared to be more closely associated with plant vigor.

Both greenbug populations and plant weights were greater in the 1957 test than in the 1956 test. This was probably due to differences in soil source and perhaps better greenhouse growing conditions.

Field Experiment.—The results of the field work are presented in table 3. Analysis of these data showed that greenbugs per gram of foliage differed significantly. They varied inversely with the amount of nitrogen applied to the wheat; which is in agreement with Arant & Jones (1951) and the results of the greenhouse experiments reported in this paper. Although greenbug populations per row-foot were highest where the most nitrogen was applied, there were less greenbugs per gram of foliage on the same treatment.

Foliage weights were significantly higher where nitrogen and phosphorus were applied in combination. The weights from the nitrogen-phosphorus combination treatment of 160 and 80 pounds per acre, respectively, were greater than the weights from the 80-pound combination. When phosphorus was used alone foliage weights were as low as those on the untreated plots.

The field results are indicative that reduced greenbug damage and less greenbugs per gram of plant are positively associated with plant size and vigor.

Table 2.—Mean green weights in grams of infested and noninfested wheat plants.

Treatment, Pounds per Acre				Noninfested			Infested		
N	P_2O_5	K_2O	$CaSO_4$	1956	1957	Mean	1956	1957	Mean
0	0	0	0	1.17	1.88	1.52	0.48	0.74	0.61
0	60	0	0	1.15	1.45	1.30	.41	.47	.44
60	0	0	0	1.16	1.78	1.47	.61	.94	.77
60	60	0	0	1.33	1.87	1.60	.61	1.18	.89
60	60	60	0	1.34	1.99	1.66	.58	1.05	.81
60	0	0	1000	1.31	1.98	1.63	.43	.64	.53

Table 3.—Mean green foliage weights in grams, number of greenbugs per row foot and number of greenbugs per gram of foliage, field experiment, 1957.

Treatment, Pounds per Acre			Foliage Weights per Foot of Row	Greenbugs per Foot of Row	Greenbugs per Gram of Foliage
N	P_2O_5	K_2O			
0	0	0	25	819	34
0	80	0	22	803	37
80	0	0	38	652	17
80	80	0	54	953	18
160	80	0	99	1040	11

SUMMARY.—Greenhouse and field tests were made to determine the effects of fertilizers on greenbug (*Toxoptera graminum* (Rond.)) infestations in wheat. In the greenhouse studies, different nutrient combinations of nitrogen, phosphorus, potash and calcium were added to soil which had been placed in 8-inch pots. Westar wheat grown in the treatments was infested with greenbugs. Population counts and green plant weights were later taken.

In the field a greenbug infestation developed on a fertility trial planted to Comanche wheat. Fertilizer applications were made in September, one day before seeding. Greenbug counts and foliage clippings were made April 1.

In the greenhouse test fewer greenbugs per gram of foliage, and also less plant damage, occurred where nitrogen was included in the nutrient combinations with the exception of the nitrogen-calcium combination. Field counts showed greenbugs highest, per linear row-foot, on the heaviest nitrogen treatment but least per gram of foliage. Greenbugs per gram of plant varied inversely with the amount of nitrogen applied. Plant growth was better when phosphorus was used in combination with nitrogen.

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Effect of Hot-Water Treatment for Hard Cottonseed on a Pink Bollworm Infestation[1]

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In certain of the new cotton varieties many of the seeds germinate slowly and erratically. Such seeds are usually called hard seeds. This poor germination causes a poor stand and nonuniformity of production unless an excess of seeds are planted, which of course is uneconomical. A hot-water treatment for reducing this problem of hard cottonseed has been developed by Walhood (1956a). This treatment consists in immersing cottonseed in hot tap water at a temperature of 160° to 185° F. for a definite period of time. According to Walhood (1956b), there is an optimum time-temperature for each seed lot which lies between 45 and 120 seconds and 160° and 185° F. The principle of this treatment is the removal or destruction of a water-soluble and/or heat-labile germination inhibitor (Walhood 1956a, 1956b, 1957). The results are permanent, and the seed can then be dried and stored for later planting. The use of the hot-water treatment raised the question as to its effect on a pink bollworm infestation.

The destruction of pink bollworms (*Pectinophora gossypiella* (Saund.)) in cottonseed by means of hot water is not a new conception. Moreland (1904), deputy director of the Cawnpore and other experimental stations in the United Provinces of India, was apparently the first to recommend it. He observed that 100% mortality was obtained by soaking cottonseed for 5 to 10 minutes in water at 130° F. Gough & Storey (1913) also found this treatment effective, but decided that it was limited in its use as they could not treat large volumes of seed. Willcocks (1916) apparently considered hot-water treatment one of the simplest methods of eradicating the pink bollworm in cottonseed.

It would simplify the quarantine problems in the movement of treated cottonseed from infested to noninfested areas if the treatment proved effective in eliminating all pink bollworms. There were two basic questions in the determination of the destruction of the infestation. First, what are the minimum temperature and time required to obtain 100% mortality of the pink bollworm? Second, are these values within the limits set by Walhood (1956b), who stated that 180° F. and 2 minutes were the maximum values feasible without possible damage to the seed embryo?

PROCEDURE.—Two varieties of cottonseed were used, D.&P.L. 15 (upland) and Pima S-1 (American Egyptian). The D.& P.L. 15 was from the College Station area of Texas and was ginned at the Brownsville laboratory, with a 16-inch roller gin. The pink bollworm infestation in these seeds after ginning was approximately 4%. The Pima S-1 was obtained from the U.S.D.A. Southwestern Cotton Ginning Research Laboratory at Mesilla Park, New Mexico. It was ginned there in a Murray 54-inch roller gin and had an infestation of approximately 0.4%.

Samples were prepared for treatment by placing either ½ or 1 pound of the seeds in plastic screen bags 8 by 15 inches made by rolling over the edges of a 18- by 16-inch piece of 20-mesh Lumite and stapling them with an office-type paper stapler. These bags had a capacity about three times that of the ½-pound sample; thus the seeds were not packed.

The samples were immersed in a water bath for a selected time and temperature with slow, continual agitation to make sure that all seeds were equally and thoroughly exposed, and then removed and allowed to cool and drain at room temperature. The times selected were 45, 90, and 120 seconds, and the temperatures 130°, 140°, 150°, 160°, and 180° F. In general, three samples of D.&P.L. 15 and one sample of Pima S-1 cottonseed were treated for each time interval within each temperature, or 6 pounds per selected temperature. Only one 0.1-pound sample (100 double seeds containing 100 larvae) of D.& P.L. 15 cottom was treated for 45 seconds at 150°.

The treated samples were held between 7 and 14 days in the laboratory emergence room, which was controlled at 82° F. and 90 to 99% relative humidity. After the holding period, the seeds were examined by cutting through each seed at least once. Worms not obviously dead were examined closely under a 14× hand lens for mandibular movement, heartbeat, or some other sign of life.

The water bath used for most of the treatments was a Precision Scientific Company Model 161, which is controlled to ±0.02° F., but for treatment at the two highest temperatures a 10-gallon water bath having a controlled temperature of ±0.2° was assembled at the laboratory. An Alinco Model P-102 potentiometer with copper-constantan thermocouples was used in making all temperature measurements.

Temperature data were obtained on single solid seeds by inserting a small thermocouple into a hole bored into the center of the seed and sealing it in with cement. On pink bollworm damaged seeds the thermocouple was simply inserted in the entrance hole.

Germination was obtained by placing damp seeds on wet blotting paper in covered Petri dishes and holding them at room temperature. Water was added if any dryness became evident. Mold was controlled by dusting with chloranil.

A total of 28.5 pounds of cottonseed was processed in these experiments. This amounted to approximately 114,000 cottonseeds containing about 3,610 diapaused pink bollworms.

RESULTS.—The results of the immersion tests are shown in table 1. Germination of all the treated seed was normal, averaging approximately 79%. The sample treated in 150° F. hot water for 45 seconds was not comparable to the others as the pink bollworms were in double seed selected from ginned seed, which meant that every seed treated contained a worm.

Temperatures between 150° and 180° F. for at least 45 seconds gave a 100% mortality. At 140° F. or below, 2

[1] Accepted for publication June 7, 1957.
[2] In cooperation with the Texas Agricultural Experiment Station.
[3] Various members of the staff of the Brownsville, Tex., laboratory assisted in this study.

Table 1.—Effect of heat treatment on pink bollworms infesting cottonseeds.

Time (Seconds)	Temperature (° F.)	Per Cent Mortality	
		Pima S-1 (15 Worms)	D.&P.L. 15 (250 Worms)
45	130	71.4[a]	85.3
	140	100	99.7
	150	—	100[b]
	160	100	100
	180	100	100
80	130	85.8	99.5
	140	100	100
	160	100	100
	180	100	100
120	130	71.5	97.0
	140	100	100[c]
	160	100	100[d]
	180	100	100

[a] 45 worms.
[b] 100 double seeds containing 100 worms.
[c] 375 worms.
[d] 525 worms.

minutes was not sufficient to give complete kill. In general, these results are similar to those obtained by Bulger (1945) while determining the value of dry and steam heat as a pink bollworm eradication agent in cottonseed. This is particularly true of his results using steam heat.

The results of the individual seed tests are shown in figure 1. It took 45 seconds for the normal linted D.& P.L. 15 seed and 35 seconds for Pima S-1, which usually has no lint, to reach the ambient temperature of 130° F. from room temperature (82°). The rise in temperature in solid seed was apparently constant and amounted to 0.93° per second in D.&P.L. 15 seed and 1.2° in Pima S-1. Apparently it will always take about the same time to reach a given temperature and that time would be dependent upon the initial room temperature and the amount of lint, and independent of the maximum temperature applied within the limits tested here. Linted cottonseeds subjected to prior wetting took longer to reach the ambient temperature because the cold water held in the lint acted as insulation. Seeds that were hollow or infested with a pink bollworm took from 10 to 40 seconds, depending upon both the conducted heat and the speed which the water itself could penetrate.

The data indicate that it takes, at most, approximately 45 seconds for the heat to penetrate to the center of the seed and subject the pink bollworm to a lethal temperature, if the seed and the enclosed worm are considered as a solid seed. If the hollowed area created by the pink bollworm is large enough to allow fast penetration of the hot water, less time is required.

SUMMARY.—One-half-pound samples of cottonseed infested with the pink bollworm (*Pectinophora gossypiella* (Saund.)) were immersed in hot tap water at temperatures of 130° to 180° F. for intervals of 45 to 120 seconds. There was a total destruction of the infestation at 150° F. or higher for 45 seconds. It took 45 seconds for the center of a solid linted D. & P.L. 15 and 35 seconds for that of a solid delinted Pima S-1 cottonseed immersed in hot water to reach an ambient temperature of 130° F.

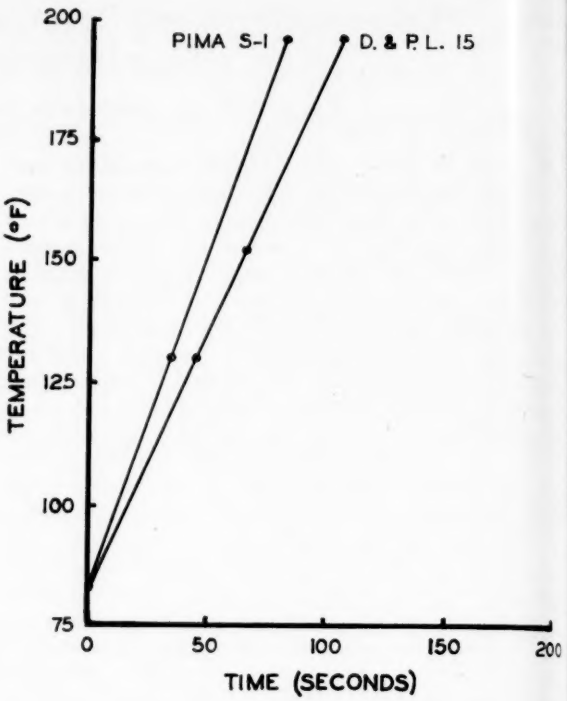

FIG. 1.—Heat penetration of solid cottonseeds.

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Insecticidal Control of the Carrot Weevil in Canning Carrots[1]

J. M. Wright[2] and G. C. Decker[3]

During the summer of 1947, canning carrots grown on sandy soil near Lansing and Blue Island, Illinois, became moderately infested with the carrot weevil, *Listronotus oregonensis* (LeC.). Damage the following year was severe throughout the large contract carrot acreage in south Cook County. Although the carrot weevil had been reported injuring carrots in the East St. Louis, Freeport, Peoria, and Quincy, Illinois areas as early as 1923, its invasion of the large northern Illinois acreage made it a major vegetable pest and in the spring of 1949 a project to develop satisfactory control methods of the weevil was begun. The study was conducted in the South Holland, Illinois, area because of the severity of the weevil infestation there and the large acreage affected. Carrots are the only commercial crop attacked by the carrot weevil in Illinois, although larvae have been collected from home garden patches of celery and curly-leaved parsley throughout most of the state.

Few suggestions for control of this insect are to be found in the literature. Chittenden (1909) recognized the possibilities of crop rotation but also suggested the use of carbon bisulphide or kerosene emulsions applied to the roots of parsley plants. Boyce (1927) felt that rotation was apparently the only effective control measure on Long Island. Pepper & Hagmann (1938) showed that calcium arsenate dust and an apple-sodium fluosilicate bait offered some possibilities. Pepper (1942) obtained good weevil control on celery, carrots and parsley using a 95 to 5 apple pomace-calcium arsenate bait applied three to four times at the rate of 40 to 50 pounds per acre.

Hill (1948) did not consider 3% DDT dust effective, possibly due to improper timing. DDT and BHC were found to be effective by Bare (1950), however, but methoxychlor, chlordane and toxaphene gave poor control. Recently, Semel (1957) reported that dieldrin and heptachlor as emulsion sprays and broadcast treatments gave excellent control of the carrot weevil in parsley and also found chlordane and toxaphene to be decidedly inferior.

Life History and Habits.—The carrot weevil overwinters in the adult stage. Favorite overwintering quarters in the South Holland area are grassy roadsides, ditchbanks and fencerows close to previously infested fields. Emergence from hibernation takes place about the first week in May in northern Illinois. Feeding begins immediately. Eggs are deposited in small cavities which the females chew in the stems, crowns, or exposed roots of the plants. The first eggs are laid about the last week in May. First-generation adults appear from mid-July to early August and a second generation is completed by late September and early October. These adults hibernate over winter.

Although the beetles possess wings they apparently seldom fly. The authors have not seen carrot weevils in flight. Pepper (1942) reported having captured about 10 adults on the wing over a period of 5 years. The way infestations develop indicates that the beetles migrate on foot rather than by flying. Infestations early in the season are observed mostly in the rows nearest the hibernation quarters. Later in the season gradual migration within the fields takes place and the infestations become more general.

Soil Treatments.—*Methods.*—When the present study began there was an urgent need for satisfactory control methods. Carrot growers in the South Holland area had made a practice of rotating fields as much as possible within the limits of their relatively small farms but found this useless against the weevil. Since the larva feeds underground and pupates in the soil, it was felt that a soil treatment might be the means of weevil control. Accordingly, in the spring of 1949 soil treatments were applied to plots on two farms where serious infestations had occurred in 1948.

Plots on the Dusseljee farm were 8 rows wide and 20 rods long while plots on the DeBoer-Murray field were 8 rows wide and 10 rods long. Both fields had been plowed the previous fall. On April 12, prior to final disking, identical treatments of chlordane at rates of 10 pounds and 5 pounds per acre and parathion at rates of 1 pound and ½ pound per acre were applied to the surface of the seedbed of each field. The plots were disked and planted immediately after application of the insecticides. Treatments were replicated four times in a randomized block design in each field.

Infestation counts in four 10 foot sections from the four center rows of each plot were made October 18.

Results.—Soil treatments applied in this test did not control the carrot weevil. On the DeBoer-Murray field both parathion treatments resulted in slightly lower infestations although differences were not significant. On the Dusseljee field, all treatments gave higher infestations than were found in the untreated check, table 1.

Table 1.—Results of pre-planting soil treatments for control of the carrot weevil. South Holland, Illinois. 1949.

Treatment	Pounds Toxicant per Acre	Per Cent Weevil-Damaged Carrots	
		Dusseljee Field	DeBoer-Murray Field
Chlordane	10.0	73.1	50.1
	5.0	54.7	50.1
Parathion	1.0	60.4	46.4
	.5	60.7	41.7
Check		52.3	48.6

Foliage Sprays.—*Methods.*—Since soil treatments such as were made in 1949 were not effective against the carrot weevil, several insecticides were tested as foliage sprays attempting to kill the adult weevils before oviposition took place, These tests were conducted in 1950, 1951 and 1952. During this period aldrin, chlordane,

[1] Data taken from a thesis presented by the senior author to the Graduate School of the University of Illinois in partial fulfillment of the requirements for the Doctor of Philosophy Degree. Accepted for publication June 14, 1957.

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DDT, dieldrin, heptachlor, lindane, methoxychlor, parathion, and toxaphene were tested. In all 3 years the plots were 4 rows wide and 20 feet long with all treatments being replicated three times. The materials were applied with a knapsack sprayer at the rate of 125 gallons of spray per acre. The spray was directed against the stems of the plants. The first of four applications each year was timed to coincide with the earliest oviposition. In 1950, sprays were applied May 23, June 5, June 14, and June 21. Dates of application in 1951 were June 1, June 7, June 14, and June 25, and in 1952 were June 5, June 18, June 26, and July 3.

Infestation records were made by counting the number of damaged roots in 25 plants selected at random from the two center rows of each plot. These records were made July 24, 1950, August 1, 1951, and July 15, 1952.

When it appeared in the 1950 plots that DDT was giving good control of the weevil, a test comparing DDT with the apple pomace-calcium arsenate (95 to 5) bait as recommended by Pepper was made in a small field adjacent to the plots. Treatments were DDT at 2 pounds per acre, DDT at 1 pound per acre, DDT 1 pound plus bait at 40 pounds per acre, and bait at 40 pounds per acre. Each treatment occupied eight rows the length of the field and treatments were not replicated. Two applications of bait had already been made on this field so that the DDT and bait treatments in the test were in addition to these. The check plot received no further treatment. Two applications of DDT and bait were made, the first on June 22 and the second on July 12. The DDT was applied in emulsion form with a tractor-mounted power take-off sprayer delivering 50 gallons of spray per acre. The bait was broadcast by hand.

Infestation counts of the number of damaged roots in 25 consecutive plants at each of four places in each plot were made on July 25 at the end of first generation and again on October 3 during harvest of the field.

Trials using DDT at the rate of 1½ pounds per acre were conducted in 1952 to determine the number of applications necessary for satisfactory control of the weevil. Although DDT had been tested in small plots only at a rate of 1 pound per acre it was decided to increase the rate of application in these tests since the heavier dosage is recommended for control of the six-spotted leafhopper, *Macrosteles fascifrons* (Stål), the vector of aster yellows in carrots. Plots were 4 rows wide and 250 feet long. All treatments were applied with the same sprayer as was used in the 1950 DDT-bait tests. Treatments were replicated three times. Schedules of one to eight applications, with the first application timed to coincide with the earliest oviposition, were carried out as shown in table 4.

On September 23, damage counts were made on 50 consecutive carrots at each of two places from the two center rows of each plot. Yields were determined by counting the number of bushels harvested from the two center rows of each plot.

Results.—Of the five materials tested in small plots in 1950, DDT gave the greatest reduction in damage. DDT was not significantly better than dieldrin but was significantly better than parathion, toxaphene or chlordane. Lindane gave the greatest reduction in injury in the 1951 tests although there were no significant differences between lindane, DDT and parathion. Lindane was, however, significantly better than dieldrin and aldrin. In the 1952 tests DDT again gave the greatest reduction in injury but was not significantly better than lindane. Differences between DDT and chlordane, methoxychlor and heptachlor were significant. Results for all three tests are shown in table 2.

In the DDT-bait comparison, damage counts made on July 25 at the end of first generation revealed a decided superiority for the DDT treatments. DDT at 2 pounds per acre and DDT at 1 pound plus bait at 40 pounds per acre gave the greatest reductions in injury. Bait alone was inferior. Records made on October 3 showed that the bait alone gave almost no control, while the DDT treatments had maintained their high levels of control. DDT at the rate of 2 pounds per acre still gave the greatest reduction in injury, followed closely by DDT plus bait and DDT at 1 pound per acre (Table 3).

On canning carrots, which are planted early in the season and not harvested until rather late and are thus subjected to prolonged attack by the carrot weevil, it was apparent that the bait did not give a high enough initial kill nor did it give protection for a long enough period of time. Results of trials comparing different schedules of applications are given in table 4.

Good weevil control was obtained from all schedules

Table 2.—Results of three years' tests with different insecticides for control of the carrot weevil. South Holland, Illinois, 1950-51-52.

Treatment	Pounds Toxicant per Acre	1950		1951		1952	
		Per Cent		Per Cent		Per Cent	
		Damaged Carrots	Reduction in Injury	Damaged Carrots	Reduction in Injury	Damaged Carrots	Reduction in Injury
Aldrin	0.5			42.7	28.8		
Chlordane	1.0	58.7	29.0			21.3	54.4
DDT	1.0	17.3	79.1	8.0	86.7	6.7	85.7
Dieldrin	.5	26.7	67.7	18.7	68.8		
Heptachlor	.25					41.3	11.6
Lindane	.25			4.0	93.3	10.7	77.1
Methoxychlor	1.5					30.7	34.3
Parathion	.3	48.0	42.0	9.3	84.5		
Toxaphene	2.0	50.7	38.7				
Check	—	82.7	—	60.0	—	46.7	—
L.S.D. at 5% level		44.0	34.2	18.3	22.4	15.0	30.9

Table 3.—Results of test comparing DDT with apple pomace-calcium arsenate bait for carrot weevil control. South Holland, Illinois. 1950.

Treatment	Pounds per Acre	July 25		October 3	
		Per Cent		Per Cent	
		Damaged Carrots	Reduction in Injury	Damaged Carrots	Reduction in Injury
DDT	1.0	6.0	68.4	11.0	83.1
DDT	2.0	3.0	84.2	5.0	92.3
DDT plus apple pomace-calcium arsenate bait	40.0	3.0	84.2	7.0	89.2
Apple pomace-calcium arsenate bait	40.0	13.0	31.6	59.0	9.2
Check	—	19.0	—	65.0	—

with three or more applications with the greatest reduction in weevil damage given by four applications spaced at regular intervals. Even though as many or more applications were made, weevil control from the interrupted schedules did not equal the control given by four regularly spaced applications. From these data it would appear that three applications at 7- to 10-day intervals gave sufficient control, with only 1.7% of the carrots showing damage. However, since the incidence of rot developing in damaged carrots held in storage necessitates obtaining as good control as is practically possible, the fourth application (also valuable in reducing carrot yellows) may be fully justifiable even though the apparent further reduction in damage was not significant in these tests.

The highest yield was obtained from four applications at regular intervals although only yields from the single application and two-application interrupted schedules were significantly lower. Yields correlated significantly with amount of damage ($r = -0.951$). When the regression coefficient of yield in tons per acre on per cent of carrots damaged was calculated, a significant coefficient of -0.27 was found.

SUMMARY.—Good control of the carrot weevil (*Listronotus oregonensis* (LeC.)) on canning carrots was obtained from DDT and lindane when applied as foliage sprays. Parathion and dieldrin gave fairly good results but aldrin, chlordane, heptachlor, methoxychlor, and toxaphene were inferior. Semel (1957) found heptachlor effective against the weevil on parsley but at a somewhat higher dosage than was used in the tests reported here. Parathion and chlordane soil treatments were ineffective. Apple pomace-calcium arsenate bait as recommended by Pepper (1942) did not offer high enough initial kill or sufficient residual kill of the weevil to be successful on canning carrots. Since *Macrosteles fascifrons* (Stål), vector of aster yellows of carrots, is a pest in Illinois, growers have used DDT to control both this leafhopper and the carrot weevil. Maximum practical control requires four applications of DDT at a rate of 1½ pounds per acre per application at 7- to 10-day intervals. It is necessary to make the first application when weevil oviposition begins, usually in late May, at which time the carrots are 2 to 3 inches tall. Thus timing and number of applications are identical for both pests.

Table 4.—Results of test to determine necessary number and timing of DDT applications for control of the carrot weevil. South Holland, Illinois. 1952.

Number and Time of Applications	Per Cent		Number of Bushels Yield
	Damaged Carrots	Reduction in Injury	
1 May 29	9.0	44.8	26.0
2 May 29, June 6	5.3	67.5	27.3
3 May 29, June 6, 16	1.7	89.6	30.0
4 May 29, June 6, 16, 24	.3	98.2	31.3
2 May 29, July 1	11.3	30.7	24.7
4 May 29, June 6, July 1, 9	1.3	92.0	28.0
6 May 29, June 6, 16, July 1, 9, 15	2.3	85.9	28.7
8 May 29, June 6, 16, 24, July 1, 9, 15, 23	0.0	100.0	29.3
Check	16.3	—	21.7
L.S.D. at 5% level	4.0	17.4	5.0

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Systemic Insecticides Applied in Low- and High-Gallonage Sprays on Strawberry, Lima Bean, and Swiss Chard[1]

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When demeton was released for the control of strawberry pests in 1955 in California, the label directions stipulated that it be applied in 100 gallons of water per acre. Some county officials interpreted this literally and would not issue permits for its use unless it was to be applied in this manner. However, most growers were equipped with low-gallonage sprayers, and they did not wish to convert their machines for high-gallonage spraying because of the added expense, time, and labor required to spray by this method. Furthermore, it was necessary to consider the hazard to the applicator, who is exposed to the systemic for a longer time with high-gallonage sprays.

Up to this time all our tests with systemic insecticides on strawberries and other crops had been conducted with low-gallonage sprays. In 1955 a series of tests were conducted with different systemic insecticides applied in both low- and high-gallonage sprays.

These tests were conducted in southern California on small plots 15 to 40 feet long and one row or bed wide ($2\frac{1}{2}$ to $3\frac{1}{2}$ feet), arranged in randomized blocks. The applications were made with a high-pressure mist sprayer at about 5 and 10 gallons, and with a knapsack sprayer at about 50 and 100 gallons per acre. Tests on strawberries and lima beans were for the control of the two-spotted spider mite (*Tetranychus telarius* (L.)), and on swiss chard for the control of the green peach aphid (*Myzus persicae* (Sulz.)), the bean aphid (*Aphis fabae* Scop.), and *Macrosiphum* sp.

Small plots were used because of the small amount of material available and the necessity of destroying the crop thus treated. It was surprising to find that significant differences were obtained where plots were so small and close together, especially since very toxic insecticides were used. The test on lima beans was conducted on single rows $2\frac{1}{2}$ feet apart, but significant differences in the amount of leaf surface damaged by mites were apparent nearly 2 months after treatment.

The first test on strawberries was on second-year berries, and the applications were made on March 14. There were four replicates. Counts were made on five leaflets per plot on March 21, 28, April 5 and 12, and table 1 gives the averages of these counts. All materials were more effective than Guthion. Methyl demeton, demeton, and schradan held the population below five mites per leaflet for more than 4 weeks, and were more effective than Thimet, Am. Cyanamid 12008, and Pyrazoxon (Diethyl 5-(3-methylpyrazolyl)phosphate). However, smaller dosages were used of the last three materials. Low-gallonage sprays tended to be better than high-gallonage sprays.

The second test on strawberries was made on a new planting, on plots 30 feet long, with six replicates. Applications were made on April 1 at 5 and 50 gallons per acre. Three counts of five leaflets per plot were made on April 6, 14, and 27. There were no check plots in this test, but the control with the different materials was essentially the same as in the first test. Guthion was inferior to all others; and demeton, methyl demeton, and schradan were the only materials to hold the population at less than five mites per leaflet for the 4-week period. Amounts of toxicant applied per acre were almost equal for the two methods. Results with high-gallonage sprays tended to be better than with low-gallonage sprays.

Table 1.—Control of the two-spotted spider mite on strawberry with low-gallonage sprays at the strengths given, and high-gallonage sprays at one-tenth these strengths.

Insecticide and Pounds per Gallon of EC	Strength of Low Gallonage Spray	Number of Mites per Leaflet				Average
		Low-Gallonage Spray		High-Gallonage Spray		
		5 Gal./A	10 Gal./A	50 Gal./A	100 Gal./A	
Methyl demeton 5	1- 49	2.9	1.0	1.2	1.0	1.5
Demeton 5	1- 39	6.3	1.0	1.0	.6	2.2
Schradan 6.5	1- 31.5	4.3	1.2	5.2	.5	2.8
Thimet 4	1-159	7.4	2.7	7.6	4.8	5.6
Am. Cyanamid 12008, 4	1-159	8.8	5.5	6.5	4.5	6.3
Pyrazoxon 2	1- 79	9.4	5.8	11.9	4.5	7.9
Guthion 2	1- 39	25.1	21.9	35.6	35.4	29.4[a]
Check						32.9[a]
L.S.D. at 5% level						2.2

[a] Not included in the analyses for L.S.D.

On lima beans the plots were 38 feet long, one row wide with rows $2\frac{1}{2}$ feet apart, and were replicated six times. The applications were made on June 2, and counts on ten half-leaflets per plot were made on June 17 and July 7. The percentage of leaf surface damaged by mites was determined on 10 leaflets per plot on July 28 and 29. Differences were highly significant, and followed the same order as in the mite counts. In mites per leaflet (table 2) the treatments and materials were similar in effectiveness except that the high-gallonage Guthion spray was inferior.

On swiss chard the plots were 42 feet long, one bed wide

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Table 2.—Control of the two-spotted spider mite on lima beans with low-gallonage sprays at the strengths given, and high-gallonage sprays at one-tenth these strengths.

Insecticide and Pounds per Gallon of EC	Strength of Low-Gallonage Spray	Number of Mites per Leaflet		
		Low-Gallonage Spray (5 Gal./A)	High-Gallonage Spray (50 Gal./A)	Average
Demeton 2	1–19	0	0.1	0
Methyl demeton 5	1–24	.1	.2	.2
Thimet 4	1–39	.2	.2	.2
Pyrazoxon 2	1–19	.5	.0	.3
Am. Cyanamid 12008, 4	1–39	.0	.8	.4
Schradan 6.5	1–63	.3	.4	.4
Guthion 2	1–9	.9	4.2	2.5
Check	—	—	—	7.8[a]
L.S.D. at 5% level				1.5

[a] Not included in the analysis for L.S.D.

Table 3.—Control of aphids on swiss chard with low-gallonage sprays at the strengths given, and with high-gallonage sprays at one-fifth these strengths.

Insecticide and Pounds per Gallon of EC	Strength of Low-Gallonage Spray	Number of Aphids per Leaf		
		Low-Gallonage Spray (10 Gal./A)	High-Gallonage Spray (50 Gal./A)	Average
Chemagro 103, 2	1–79	0.7	0.3	0.5
Chemagro 104, 2	1–79	.6	.6	.6
Chemagro 102, 2	1–79	.7	.5	.6
Schradan 6.5	1–64	.6	1.0	.8
Demeton 2	1–79	1.2	.6	.9
Isolan 2	1–79	1.4	1.2	1.3
Pyrazoxon 2	1–79	1.5	1.2	1.4
Thimet 4	1–79	1.9	1.6	1.8
Guthion 2	1–19	5.9	6.6	6.2[a]
Check	—	—	—	3.7[a]
L.S.D. at 5% level	—	—	—	.7

[a] Not included in the analysis for L.S.D.

(double row of plants on each bed) with beds 3 feet apart, and the plots were replicated five times. Applications for Aphid control were made at 10 and 50 gallons per acre. The first application was made on October 20 when the plants were in the 4-leaf stage, and a second on November 9 when they were in the 6- and 8-leaf stages. In a count made on November 7 on 10 leaves per plot, control was less than 50% at the best, but the low-gallonage spray was significantly better than the high-gallonage spray. After the second application two counts were made on five leaves per plot, on November 22 and 30 (table 3). Guthion gave no control and was not included in the analysis. Formulations of the Chemagro compounds 102 (*S*-2(ethylthio)ethyl 0,0-dimethyl phosphorothioate), 103 (*S*-2-(ethylsufinyl) ethyl 0,0-dimethyl phosphorathioate), and 104 (*S*-2-(ethylsulfonyl)ethyl 0,0-dimethyl phosphorothioate) were more effective than demeton, Isolan, Pyrazoxon, and Thimet. High-gallonage sprays tended to be better than low-gallonage sprays.

These treatments on strawberries, lima beans, and swiss chard caused no phytotoxicity. On certain crops, such as cabbage, where burn has resulted from applications of demeton in low-gallonage sprays, high-gallonage sprays might be advantageous, although it would probably not be necessary to apply as much as 100 gallons per acre, in order to avoid plant injury.

Response of Insects to Different Sources of Black Light[1]

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During the last few years various workers have studied the response of insects to black light and white light. This paper reports on a study at Tallulah, Louisiana, in 1955 and 1956 in which three sources of black light were compared for their attraction to certain species of Lepidoptera.

Early work indicated that light traps were not very satisfactory as a means of collecting insects. Frost (1952) made a study of the light traps used prior to 1950, and decided that the poor results were obtained because suitable light sources were not available.

Burks *et al.* (1938) reported that mercury-vapor light was superior in many respects to other lights for use in attracting nocturnal insects. According to Martin & Houser (1941), *Heliothis zea* (=*armigera*) (Boddie) and *Pseudaletia* (=*Cirphis*) *unipuncta* (Haw.) showed no sharp preference for any of a series of lights ranging in brightness from 2,300 to 65,000 lumens with the exception of a 150-watt tungsten light. Gui *et al.* (1942) found that insects were attracted to lights of various colors, but that blue light was the most effective. Frost (1953, 1954) compared black and white lights of equal wattage and reported that Microlepidoptera were more strongly attracted to the black light but Macrolepidoptera were about equally attracted to both lights. Pfrimmer (1955) reported that a trap containing a BLB fluorescent lamp attracted nearly 2½ times as many Lepidoptera as one with a BL fluorescent lamp, but that some individual species showed a preference for the BL lamp. Merkl & Pfrimmer (1955) compared the catches of 13 species of Lepidoptera in mercury-vapor and black-light traps, and found that 10 of the species were caught in greatest numbers in the black-light trap.

Herms & Ellsworth (1934) found that 45.5% of the codling moths caught in a trap containing a blue light were females, but only 25.8% of those caught in a trap using a white light. According to Stirrett (1938), European corn borer males were attracted to lights in larger numbers than were females. Ficht & Hienton (1939) reported that 56.9% of the European corn borer moths captured in their light traps in 1937 and 65.5% of those captured in 1938 were females. Pfrimmer *et al.* (1955) stated that only 26% of the pink bollworm moths attracted to black lights were females.

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[2] In cooperation with the Agricultural Engineering Research Division.

METHODS AND MATERIALS.—Three light traps were operated at Tallulah. Two of them were Minnesota-type traps of similar design, and constructed from galvanized sheet metal with a 15-watt fluorescent lamp as the source of light in each—a BL in one and a BLB in the other. Each lamp was mounted vertically in the center of the trap. The third trap contained a 100-watt mercury-vapor lamp of the spot type, described by Glick & Hollingsworth (1954). The spectral-energy distribution of the BL and BLB lamps has been given previously (Pfrimmer 1955).

The BL and BLB lamps were installed as new lamps on March 16, 1955. On January 26, 1956, they were replaced with new lamps, and the position of the two traps were reversed. These lamps were operated continuously after they were installed. The mercury-vapor lamp was one that had been in use since 1953. It was turned on when the collection jars were placed on the traps in the evening and off when the jars were removed in the morning. In 1955 the traps were operated daily from March 16 to September 10 and intermittently from September 24 to December 15; in 1956 they were operated intermittently from January 26 to March 19, daily from March 23 to August 6, and intermittently from August 17 to September 20. Potassium cyanide was used as the killing agent in the collection jars.

RESULTS.—A summary of each year's collections of certain species of Lepidoptera is shown in table 1. It will be noted that the combined collections of most of the species were considerably smaller in 1956 than in 1955. For some species this may be due partially to the traps not being operated as much in the fall of 1956. Another possible reason might be the widespread use for the first time of a phosphorus insecticide for the control of cotton insects during 1956. After applications of this material, many dead moths were noted in the field. An exception to the decrease in numbers is observed in *Loxostege similalis* (Guen.). Although the mercury-vapor trap caught fewer *similalis* moths in 1956, the total collection for the three traps was nearly twice as great as in 1955.

Most of the species responded in greatest numbers to the BL trap both years. The mercury-vapor trap caught the most of *Celerio lineata* (F.) and *Protoparce quinquemaculata* (Haw.) in 1955, and of *P. quinquemaculata* in 1956. The BLB trap did not rank first in the collection of any species in 1955, but it caught the greatest numbers of five species in 1956. The location of the traps may have had some influence on the response of the moths. Despite the reversal of the BL and BLB traps in 1956, more of the Lepidoptera were attracted by the BL lamp both years. The BLB trap caught 34% of the total in 1955 and 43% in 1956. The mercury-vapor trap caught 18% in 1955 and dropped off to only 9% in 1956.

Most of the species recorded in table 1 were sexed. The percentages of females caught in each trap are shown in table 2. All traps had more species that were predominantly females in 1956 than in 1955. In 1955 the BLB trap had the most species that were predominantly females, and the BL trap had the fewest. In 1956 the BL and mercury-vapor traps were tied for the most species predominantly female. In all traps in both years *Laphygma frugiperda* (A. & S.) and *Pseudaletia unipuncta* (Haw.) were over 50% females, and *Protoparce sexta* (Johan.), *Estigmene acrea* (Drury) and *Prodenia ornithogalli* Guen. were over 50% males.

Merkl & Pfrimmer (1955) found a highly significant correlation between the number of bollworm moths caught in light traps and the number of bollworm eggs per 100 terminals found in cotton fields during the summer of 1954. In 1955 and 1956 the numbers of bollworm moths caught in light traps at Tallulah were compared with the number of bollworm eggs found in cotton fields in the area. In 1955 the numbers of moths trapped showed no significant correlation with the number of eggs found in the same week, but the correlation with the number of eggs found in the following week was significant. If this were true every year, or even in most years, the light trap would be a very valuable tool for predicting when bollworm infestations might develop. However, in 1956 the correlation was not significant between the number of moths trapped and the number of eggs found either in the same week or the following week.

Table 1.—Catches of some species of Lepidoptera in three light traps at Tallulah, La.

SPECIES	1955			1956		
	Mercury-Vapor	BL	BLB	Mercury-Vapor	BL	BLB
Agrius cingulatus (F.)	93	114	101	1	19	15
Agrotis malefida Guen.	38	252	139	17	129	128
Agrotis ypsilon (Rott.)	201	1,277	877	59	326	440
Celerio lineata (F.)	174	110	144	27	82	28
Estigmene acrea (Drury)	18	167	114	8	56	58
Feltia subterranea (F.)	198	1,810	1,042	117	1,006	886
Heliothis virescens (F.)	144	391	202	59	175	181
Heliothis zea (Boddie)	1,031	7,792	4,587	455	3,266	2,176
Laphygma exigua (Hbn.)	543	2,245	1,887	224	1,108	617
Laphygma frugiperda (A. & S.)	185	710	564	61	278	260
Loxostege similalis (Guen.)	2,941	7,259	5,975	2,564	14,116	13,278
Peridroma margaritosa (Haw.)	82	424	222	69	329	352
Prodenia ornithogalli Guen.	499	2,522	1,781	184	1,213	1,294
Protoparce quinquemaculata (Haw.)	51	40	38	12	11	3
Protoparce sexta (Johan.)	202	512	367	111	598	251
Pseudaletia unipuncta (Haw.)	2,119	4,328	3,069	283	1,279	1,275
Trichoplusia ni (Hbn.)	1,345	1,568	919	61	134	120

Table 2.—Percentage of females of some species of Lepidoptera collected in three light traps at Tallulah, La.

Species	1955			1956		
	Mercury-Vapor	BL	BLB	Mercury-Vapor	BL	BLB
Agrotis malefida Guen.	69	48	53	65	55	55
Agrotis ypsilon (Rott.)	49	50	57	56	52	60
Celerio lineata (F.)	55	62	62	44	70	57
Estigmene acrea (Drury)	11	7	12	13	16	9
Feltia subterranea (F.)	55	40	46	36	39	38
Heliothis virescens (F.)	47	47	46	58	54	50
Heliothis zea (Boddie)	50	49	53	54	55	49
Laphygma exigua (Hbn.)	30	36	38	63	59	55
Laphygma frugiperda (A. & S.)	68	63	72	80	83	69
Peridroma margaritosa (Haw.)	49	50	48	51	56	58
Prodenia ornithogalli Guen.	23	28	32	28	32	22
Protoparce quinquemaculata (Haw.)	41	45	45	58	55	33
Protoparce sexta (Johan.)	40	22	33	41	29	20
Pseudaletia unipuncta (Haw.)	51	56	55	64	75	81
Trichoplusia ni (Hbn.)	37	33	39	51	39	38

Much valuable information has been gained in recent years on the use of light traps as survey tools, but a great deal more work needs to be done. It is already apparent that different survey problems require different trap designs and light sources for best results.

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Chemical Control of *Tetranychus telarius* (L.) and *T. cinnabarinus* (Bois.) on Cotton[1]

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The most prevalent species of spider mites attacking cotton throughout North Carolina in 1955–56 were the two-spotted spider mite, *Tetranychus telarius* (L.), and *T. cinnabarinus* (Bois.) (Farrier 1955–56). Other species recorded on cotton in the state included the strawberry spider mite (*T. altanticus* McG.), the tumid spider mite (*T. tumidus* Banks), the Schoenei spider mite (*T. schoenei* McG.), and desert spider mite (*T. desertorum* Banks.)

Tetranychus telarius and *T. cinnabarinus* are the most difficult species of spider mites to control on cotton (Anonymous 1955). Acaricides which are effective against these two species are also effective against most of the other species attacking cotton. However, certain acaricides that are effective against several of the other species are only partially effective or ineffective against *T. telarius* and *T. cinnabarinus.*

Iglinsky & Rainwater (1954) have shown that at a temperature of 88.3° F. and 45.3% relative humidity the time from egg to adult averaged 6.6 days, ranging from 5.1 to 8.8 days, for *T. bimaculatus* Harvey (*T. telarius*

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complex as resolved by Boudreaux [1956]) on cotton. Under similar conditions Iglinsky (1950) found that adult females of *T. bimaculatus* deposited an average of 54 eggs during an average life of 9.5 days. Forty-seven per cent of the eggs were deposited during the first 3 days of adult life following a preoviposition period of 1 day.

Gast (1952–54) found that one spray application of 0.25 pound of demeton per acre was highly effective against *T. bimaculatus* (*T. telarius* complex as resolved by Boudreaux [1956]) on cotton in the Dunn-Benson area of North Carolina. It generally required two or more applications of 0.25 pound of parathion or 0.75 pound of Aramite per acre at 7-day intervals to control this spider mite. Spider mite infestations declined steadily in untreated plots in some tests. Infestations increased in other tests, and chemical control of these infestations resulted in significant increases in the yield of cotton. The causes of fluctuations in spider mite infestations were not always apparent.

Adequate control of *T. telarius* and *T. cinnabarinus* on cotton has been obtained wherever demeton spray has been used commercially according to recommendations in North Carolina. However, approximately 80% of the cotton growers in the state use dusting equipment to apply insecticides and are not equipped to spray. It has been frequently necessary for growers to use several applications of available dusts against these spider mites, and control has not always been adequate. Consequently, many cotton growers in North Carolina are in need of a highly effective acaricidal dust.

Infestations of *T. telarius* and *T. cinnabarinus* reached outbreak proportions on cotton in Cleveland county, N. C., in July 1956. An investigation was begun involving the re-evaluation of currently recommended acaricides (Aramite, parathion, demeton) and the initial testing of certain experimental materials. Results of this investigation are herein reported.

MATERIALS AND METHODS.—Dust formulations of parathion, Aramite, Guthion, Kelthane, Hercules AC-528, Phostex, Trithion, Niagara 1240 (bis (S- (diethoxyphosphinothioyl) mercapto) methane), and an emulsifiable concentrate of demeton were used in this investigation (table 1). Dusts were applied with a rotary hand duster through one nozzle per row. The emulsifiable concentrate diluted with water was applied with a compressed-air sprayer through two hollow-cone nozzles per row at the rate of 6 gallons per acre. Treatments were applied as soon as the first cotton leaves began to redden. Applications were made in early morning or evening when the air was nearly calm.

All test fields were planted about the middle of April to Coker 100 Wilt cotton and fertilized in accordance with good farm practices for the area. Chlorinated hydrocarbon insecticides were applied to these fields several times during the season to control the boll weevil. In addition, the field in which experiment 1 was located received one application of sulfur dust early in the season, and one application of parathion dust 1 week prior to the beginning of the test.

In each experiment certain dusts were included in a randomized block design. Demeton and the check were included around the exterior of this design and were separated from one another and from the basic design by untreated buffer areas. Each treatment was replicated three times. Plot size varied slightly with the different experiments; but, in general, each plot was 4 rows wide and 60 feet long (1/60 acre). In each experiment the blocks were arranged side by side along the border of a cotton field. Experiments 1, 2, and 3 bordered an apple orchard, a permanent pasture, and a highway, respectively. Experiment 1 was conducted while the cotton was fruiting vigorously, experiment 2 as blooming terminated, and experiment 3 as the crop matured.

Table 1.—Field experiments for chemical control of spider mites on cotton. 1956.

Chemical and Per Cent Concentration	Pounds per Acre	Pretreatment Infestation[a]	Per Cent Reduction in Infestation on Days Indicated After Treatment		
			1	5	10
July 27		Experiment 1. *Tetranychus cinnabarinus*			
Parathion, 1	27	1073	95.0	64.8	+307.2
Aramite, 3	21	1100	85.6	86.4	+247.3
Kelthane, 5	19	1009	37.8	51.1	+455.7
Hercules AC-528, 3	28	1090	53.8	47.8	+267.8
Trithion, 4	24	1082	100.0	100.0	99.7
Phostex, 5	29	1250	91.2	54.0	+295.5
Guthion, 2½	22	1147	99.8	96.8	+421.8
Demeton[b]	0.27[c]	924	99.9	100.0	99.2
Check[b]		1039	+158.9	+169.3	+351.8
August 7		Experiment 2. *T. telarius*			
Parathion, 1	24	990	91.2	+33.7	+147.8
Aramite, 3	26	1099	84.3	67.0	+62.5
Guthion, 2½	26	992	97.1	70.3	+48.5
Trithion, 4	28	1042	99.9	99.7	96.6
Demeton[b]	0.24[c]	1081	99.9	100.0	100.0
Check[b]		876	+70.1	+229.6	+912.9
August 24		Experiment 3. *T. telarius*			
Parathion, 1	26	1074	85.5	41.3	+312.5
Niagara 1240, 2	26	1023	95.0	94.5	72.5
Trithion, 2	22	1030	100.0	100.0	99.0
Trithion, 4	26	1152	100.0	100.0	100.0
Check[b]		970	+60.1	+19.9	+568.8

[a] Number of spider mites per 30 leaves (3 replicates) 6 to 12 hours before applications.
[b] Replicated outside randomized block design.
[c] Active ingredient.

Pretreatment infestation counts were made on 10 leaves per plot (usually five leaves on each of the two middle rows). All active forms of immature and adult mites were included in the records. Leaves infested with 20 to 100 mites were selected and tagged on various parts of the plants. Lenses providing magnifications up to 20 were used in making the counts. Flashlights were used whenever the sunlight was not brilliant. All post-treatment counts were made on the original 10 leaves selected in each plot. A mite was considered alive when any movement was observed in the specimen. It was frequently necessary to probe the mites to determine whether they were dead or alive.

The only appreciable amount of precipitation (0.58 inch) from July 9 to September 20 (final harvest of cotton) occurred on August 29 (fifth day of experiment 3). During this period small amounts of rain (generally less than 0.2 inch) occurred at frequent intervals, rather strong winds prevailed, and daily maximum temperatures ranged mostly from 85° to 100° F.

RESULTS.—Parathion and Aramite were initially effective in reducing spider mite infestations (table 1). However, within a few days following treatment, infestations began to increase steadily—indicating the necessity of repeated applications at short intervals. Aramite was slightly more effective than parathion, but this small difference in effectiveness did not appear to eliminate the need for repeated applications. Kelthane, Hercules AC-528, Phostex, and Guthion did not exhibit any outstand-

ing advantages over parathion or Aramite. Niagara 1240 was far superior to parathion against *T. telarius*.

In order to achieve any control which was more than transitory, it appeared necessary to reduce spider mite populations to an extremely low level for a period of time sufficient to disrupt the life cycle. One application of Trithion dust consistently resulted in control equal to that of demeton for at least 10 days after application. Both materials virtually eliminated all active forms of spider mites. Most of the few mites recorded in those plots treated with demeton or Trithion were adults. The protection afforded by either material was apparently sufficient to disrupt the life cycle of these species. Infestations did not reach original levels during the remainder of the season. The residual properties of these materials against migratory spider mites were not evaluated.

Defoliation proceeded at a more rapid rate in the untreated plots than in any of the treated plots. No additional discoloration of the foliage was noted in those plots treated with demeton or Trithion. All of the other treated plots showed considerable additional reddening of the foliage with the exception of those plots treated with Niagara 1240, which showed only a slight additional discoloration.

The only decrease in the spider mite infestations in untreated plots was recorded in experiment 3 on the fifth day of the test following 0.58 inch of rain. This infestation increased over 500% during the remainder of the test period.

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Life History and Abundance of the Spotted Alfalfa Aphid in Arizona[1]

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The spotted alfalfa aphid, *Therioaphis* (*Pterocallidium*) *maculata* (Buckton), has beome a very important pest of alfalfa in the United States since it was reported in New Mexico in 1954 (Tuttle & Butler 1954). Dickson and his coworkers (1955) made observations of damage, host plants, life history, seasonal abundance, and predator relationships of this aphid under California conditions. Other papers on this aphid have appeared (Bieberdorf & Bryan 1956, Smith *et al.* 1956, Tuttle 1956). The present paper describes studies conducted in 1955 and 1956 on the life history and abundance of the aphid under southern Arizona conditions. The life history studies were carried on under caged conditions at Tempe, Ariz., and those on seasonal abundance were conducted in the Salt River Valley.

LIFE HISTORY.—In 1955 reproduction and longevity studies were conducted in the laboratory. Individual aphids were confined in cylindrical 1×5-inch glass tubes with the tops covered with cheesecloth and the bottoms closed with cork disks. A small hole was made in the center of each disk to permit insertion of an alfalfa stem. The glass tubes were held in shallow pans of water so that the stem ends of the alfalfa would receive a sufficient amount of water to keep the foliage succulent. In 1956 additional investigations of life history, including reproduction and longevity, were conducted in a lath house. Alate and apterous females were placed singly on caged alfalfa plants grown in 5-inch pots. When the first young were born, the adults were removed with a small aspirator to allow the progeny to remain undisturbed. The nymphs were then observed daily until they matured. The new alate and apterous females were then used for the reproduction and longevity tests. The young were removed each day and records kept of the number produced throughout the life of each female.

The spotted alfalfa aphid has four nymphal instars. Data on the length of the instars are given in table 1. All the young from both alate and apterous forms matured into apterous females.

Results of studies conducted in 1955 on nymphal development during different months are given in table 2. The nymphal period varied from 5 days in June to 13 days in March. No data were available for November through February, but the periods were probably much longer during this time. The largest number of generations in a sin-

Table 1.—Length (in days) of nymphal development of spotted alfalfa aphid, August 1956.

NUMBER OF INDIVIDUALS	INSTAR				TOTAL
	First	Second	Third	Fourth	
Alate					
23	1.5	1.1	1.6	1.9	6.1
Apterous					
30	1.5	1.1	1.8	2.0	6.4

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[2] Employed by the Arizona Agricultural Experiment Station from October 1, 1955, to June 30, 1956.

[3] In cooperation with the Arizona Agricultural Experiment Station.

Table 2.—Nymphal development and number of generations of the spotted alfalfa aphid in relation to monthly temperatures, 1955.

Month	Mean Temperature (° F.)		Nymphal Period (Days)	Number of Generations
	Outdoor	Laboratory		
March	59	70	13	2
April	64	69	8	4
May	72	77	6	5
June	80	82	5	6
July[a]	87	—	6	5
August	85	86	7	4
September	81[b]	81[b]	7	4
October	74	76	9	4
Total	—	—	—	34

[a] 1956 records.
[b] Interpolated.

gle month averaged six in June. It is assumed that the smallest number occurred in January, since it was the coldest month of the year. Inasmuch as some of the data were obtained under laboratory conditions where the temperature was higher than outdoors in March, April, and May, it is probable that the actual number of generations was smaller in the field. Moreover, it is possible that one to three generations occurred each month from November through February. On this basis, at least 35 generations occurred in southern Arizona in 1955.

Data on the reproduction and longevity studies are shown in table 3. Only 28% of the alate females and 15% of the apterous females lived beyond 30 days. One alate female produced young continuously for 35 days and one apterous female 27 days. The apterous females produced 1.2 more young per day and 1.9 more young per female during life than the alate females, but their reproduction period and longevity were 4 days shorter.

Since the largest number of young produced by an aphid was 142 and the number of generations was approximately 35, the potential number of aphids produced in 1 year from a single female would be 142^{35}, or $2.1387 \times \log 10^{70}$. Obviously, the factors of environmental resistance, including natural enemies and weather, would reduce this number considerably, but their effect has not yet been fully determined.

The relation between temperature and the daily production of young is shown in figure 1. During the period of high reproduction the apterous females produced more young than the alate females on all days but one. The greatest reproductive period of each form was the first 17 days; then there was a sharp decline which continued until the adults died. Reproduction by apterous females was greatest on the sixth day and by the alate females on the 11th day. The correlation between temperature and production of young appeared to be fairly close during the first 16 days. The sudden decline after the 17th day corresponded with the drop in temperature, but the production of young did not increase upon the ensuing rise of temperature.

Table 4.—Seasonal abundance of spotted alfalfa aphid in Salt River Valley of Arizona, 1955 and 1956.

Month	1955			1956		
	Mean Temperature (° F.)	Number of Females		Mean Temperature (° F.)	Number of Females	
		Apterous	Alate		Apterous	Alate
January	48	—	—	51	1	0.1
February	51	—	—	51	.3	.01
March	59	0.4	0.02	61	.7	.01
April	64	17	2	65	3	.1
May	72	11	1	75	1	.1
June	80	8	.5	85	3	.8
July	84	3	.2	87	—	—
August	85	.2	.01	84	—	—
September	81	.2	0	83	4	.3
October	74	1	.1	68	1	.1
November	58	4	.3	57	.8	.01
December	55	3	.4	52	.4	.3
Total		47.8	4.5		15.2	1.8

SEASONAL ABUNDANCE.—Data on seasonal abundance were obtained from surveys every 10 to 14 days in selected alfalfa fields. The aphids were counted on three trifoliate leaves selected at random—one from the top, one from the middle, and one from the bottom portion of the plants—and replicated 10 times at intervals of 20 to 50 walking steps taken diagonally across the field. The aphids were counted on 30 trifoliate leaves in each field, and the average per leaf calculated. The results are shown in table 4.

In 1955 there were two peaks of apterous-female abundance, one in April and another in November. The alate female population followed a similar trend except that the fall peak occurred 1 month later in December. Aphid abundance was influenced largely by climatic conditions. The temperature during the spring and fall was very favorable, but extremely high temperatures of 100° to 110° F. during July, August, and September appeared to be detrimental. Also during this time predators and fungus diseases played a role in reducing the aphid population.

In 1956 the abundance of females fluctuated throughout the year without any definite spring or fall peak. The total number of aphids per trifoliate leaf was less than one-third as great as in 1955. However, the growers were more conscious of damage caused by this aphid, and most of them applied insecticides throughout the year for control of the pest. Such practices probably prevented

Table 3.—Reproduction and longevity of the spotted alfalfa aphid, 1956.

Individuals	Number of Young per Female				Longevity (Days)			
	Per Day		During Life		Average			Maximum
	Average	Maximum	Average	Maximum	In Reproduction	After Reproduction	Total	
Alate 61	3	10	48	142	15	3.0	18	43
Apterous 58	4	14	50	136	11	2.9	14	38

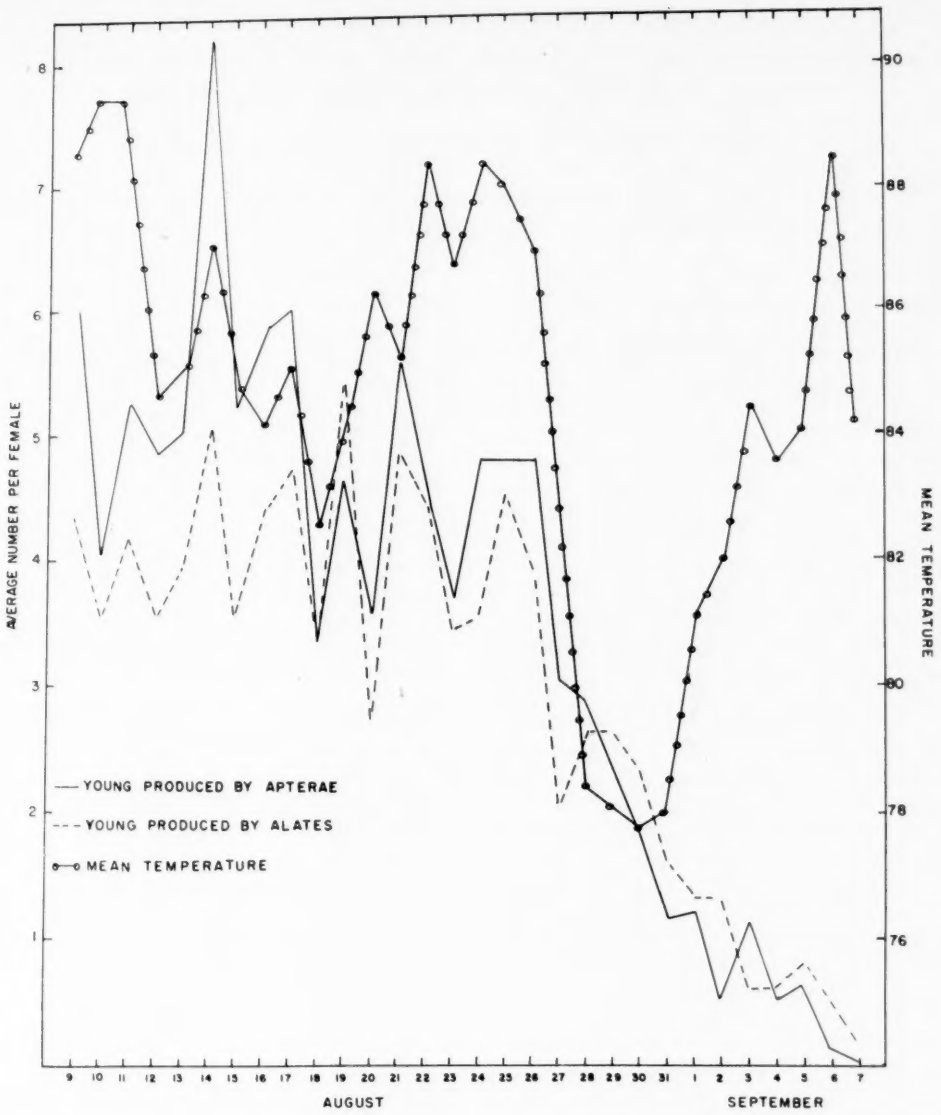

FIG. 1.—Relationship between temperature and daily production of young by 10 alate and 8 apterous females from August 9 to September 7, 1956, Tempe, Ariz.

populations from building up as they did in 1955, when there was little insecticide application.

SUMMARY.—Studies on the life history and seasonal abundance of the spotted alfalfa aphid, *Therioaphis* (*Pterocallidium*) *maculata* (*Buckton*), under Arizona conditions are described. Alate and apterous females produced progeny that had four nymphal instars. The total developmental period of the nymphs was about 6 days in August. The number of young from the alate females averaged 3 per day and from the apterous females 4. The total number of young produced by the two forms was about the same.

The reproductive potential of the aphid was calculated as $2.1387 \times \log 10^{70}$ using 35 as the approximate number of generations in one year and 142 as the highest number of young produced by a female. Reproduction was greatest in the first 16 days in adult life and was correlated with the temperature.

Two peaks of aphid abundance occurred in 1955, one in April and another in November. In 1956 definite peaks were not so evident owing to the increased use of insecticides. Temperature, predators, fungus diseases, and insecticide application were the most important factors influencing the abundance of this aphid in Arizona.

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Ineffectiveness Against Cattle Grubs of Phenothiazine and Stilbesterol in Daily Diet of Fattening Steers[1]

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During the past three decades, parasitologists have been engaged in the search for chemicals which, when administered to livestock internally, would effectively control external parasites. Although early attempts at this method of control were generally unsuccessful (Parman 1928, Bruce 1940, Babcock & Boughton 1943, Creighton *et al.* 1943), more recent work has shown that systemic insecticides are highly toxic to a wide range of ectoparasites (Lindquist *et al.* 1944, de Meillon 1946, Garnham 1947). Among the parasites susceptible to internal medication are cattle grubs, *Hypoderma lineatum* (De Vill.) and *H. bovis* (L.), which succumbed to subcutaneous injections of chlorinated hydrocarbon insecticides in peanut oil (Hoyt 1953). Cattle grubs were reported destroyed by subcutaneous and oral administration of organic compounds as well (McGregor *et al.* 1954).

The effectiveness of phenothiazine as an insecticide when administered to livestock internally was demonstrated by Knipling (1938) and Bruce (1939), who showed that when fed to cattle, the drug destroyed the larvae of the horn fly in manure, presumably because the manure contained enough of the drug to kill the larvae. Leiper (1951) subsequently observed that there was not a single case of tail-strike in lambs dropped by ewes dosed with phenothiazine 10 days prior to lambing and continued on low-level phenothiazine for 9 weeks thereafter, whereas 6 of 22 lambs dropped from untreated ewes were struck. Worden (1953) reported successful control of grubs by drenching cattle with a preparation containing phenothiazine; in one test, the medication was administered in June and again in March, whereas in the second test, the treatment was given monthly from December through March. Each dose contained, in addition to hexachloroethane and an organic tin compound, 70 grams of phenothiazine. Schwartz *et al.* (1954) observed that phenothiazine made available to cattle for prophylactic free-choice ingestion to control nematodes might prove of value also in controlling cattle grubs. The principals in these investigations consumed daily from 0 to 3 grams of phenothiazine in a salt-bonemeal-phenothiazine mixture during the grazing season, and thereafter in a grain mixture, prior to and during the period of arrival of grubs in the subcutis. It was reported that principals harbored an average of 6.5 grubs per head, as compared with 33 in the controls. Spence (1952) administered phenothiazine in doses of 7.5, 15, and 20 grams, at 15-day intervals, to calves, yearlings, and mature cattle, respectively, comprising half the members of a herd, the remainder serving as controls; he reported no difference between principals and controls with respect to incidence of *Hypoderma* larvae. The work of Cobbett *et al.* (1957) involved drenching cattle with phenothiazine at monthly intervals, at a time to coincide with the heel fly strike season; the drug was also administered daily in doses of from 2 to 8 grams, during early and late stages of larval development, for periods up to 125 days. It was concluded as a result of this work that phenothiazine as administered did not significantly influence cattle grub survival.

The data herein presented derive from the administration of phenothiazine, alone and in combination with stilbesterol, in the daily diet of fattening steers, and contribute to the evidence that the drug is ineffective as a systemic against *H. lineatum* larvae. The undertaking was prompted by interest in possible tissue deposition resulting from the use of phenothiazine and stilbesterol in combination, as well as in the effect of these drugs on weight gains in fattening steers.

PROCEDURE.—In the fall of 1955, 40 yearling Hereford steers at the University of Illinois Dixon Springs Experiment Station were removed from pasture and placed in a feed lot. All aminals had been similarly, if not identically, exposed to heel fly oviposition on open pasture during the previous spring and summer. The average weight of the steers at the start of the experiment was 670 pounds. The animals were separated into 4 groups of 10 each, in adjoining feed lots, and medication was begun on October 26, 1955. Group 1 consisted of control animals; group 2 received 10 mg. of diethylstilbesterol daily; group 3 received 2 grams of phenothiazine daily; and group 4 a combination of both drugs daily. The feed ration consisted of 2 pounds of soy bean oil meal daily, corn silage, and hammered ear corn. Phenothiazine and stilbesterol were thoroughly mixed with the oil meal, which in turn was spread over the silage. Since cattle relish both of these feeds, all was readily consumed, leaving no trace of the drugs. Feeding was continued, with no change in medication, during a 120-day fattening period.

Examination of the animals at biweekly intervals for the appearance of grubs revealed that medication was begun approximately 45 days prior to the arrival of the first few larvae in the subcutis. Administration of the drugs continued throughout the period of arrival of larvae in the backs of the animals.

On December 20, 1955, all larvae present were removed by manual expression, with the aid of Kelly forceps, from cysts in the backs of the 40 subjects. Thereafter, all encysting larvae were removed in the same manner on January 10 and finally on February 14, 1956. Extractions were so spaced that no larvae were permitted sufficient time to emerge normally.

RESULTS.—Data on the effect of phenothiazine, stilbesterol, and a combination of these drugs on the survival of *H. lineatum* larvae, in first, second, and third instars, are presented in table 1. It is apparent that the administration of 240 grams of phenothiazine in 2-gram daily doses, 1,200 mg. of stilbesterol in 10-mg. daily doses, or a

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Table 1.—**Effect of phenothiazine, stilbesterol, and a combination of both in the daily diet of fattening steers on the arrival and survival of cattle grubs in subdermal cysts.**

	Number of Live *H. lineatum* Larvae Removed from Subdermal Cysts											Total No. Dead Larvae —All Extractions
	Dec. 20, 1955				Jan. 10, 1956			Feb. 14, 1956			Totals —All Extractions	Means (Per Head)	
	Instar			Sub-totals	Instar		Sub-totals	Instar		Sub-totals			
	1	2	3		1 and 2	3		1 and 2	3				
Controls	2	61	1	64	81	10	91	3	79	82	237	23.7	6
Stilbesterol	4	27	0	31	108	7	115	9	108	117	263	26.3	7
Phenothiazine	5	29	0	34	99	11	110	16	93	109	253	25.3	5
Combination	9	28	0	37	85	5	90	7	83	90	217	21.7	2

combination of these drugs failed to halt the arrival of first instar larvae in the subcutis or to influence their survival after encystment. As demonstrated, the number of larvae reaching the subdermal location, as well as those surviving in second and third instars, is substantially the same in principals as in controls. A total of 20 dead larvae were extracted from cysts in all groups, as shown; these were found chiefly in the course of the second extraction, and almost exclusively in the second instar. The number is so small as to be of no significance, and distribution indicates that mortality was not attributable to the drugs administered.

Examination of 12 animals at autopsy, 14 days after the third and final extraction of larvae, revealed that no additional grubs arrived in the backs of these animals. It is therefore safe to assume that the 970 larvae extracted represented virtually the entire seasonal grub population for the 40 subjects.

The work of Schwartz *et al.* (1954) showed that phenothiazine administered to cattle orally had no deleterious effect on *H. lineatum* larvae that survived medication. In view of this, and the fact that all larvae in the trials herein reported survived medication, no attempt was made to investigate further the effect of phenothiazine, stilbesterol, or a combination of both on subsequent pupation and emergence of adults.

SUMMARY AND CONCLUSION.—Phenothiazine in 2-gram doses, stilbesterol in 10-mg. doses, and a combination of both were administered to young steers in the daily diet during a 120-day fattening period. Administration of the drugs began approximately 45 days prior to the arrival of *Hypoderma lineatum* (De Vill.) in the subcutis, and continued beyond the time when first instar larvae ceased to appear in dermal cysts. Extraction of all grubs from their cysts showed that these materials, as administered, did not influence the arrival of larvae in the subcutis, nor their survival while in the cysts thereafter.

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Biology and Seasonal Development of the Alfalfa Weevil in Maryland[1]

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The alfalfa weevil (*Hypera postica* (Gyll.)), which had long been an important pest of alfalfa in the intermountain region of the West, was first reported in Maryland and adjoining states in 1952 (Poos & Bissell 1953). Since that time it has continued to spread in the East and has caused great damage. The presence of this insect in an area that was generally believed unsuitable aroused several questions, one being the changes, if any, that would take place in its habits, and another whether or not it would inflict economic damage in its new environment.

This paper is an attempt to answer the former question. The latter question has been most emphatically answered during the last 4 years. The necessity for treatment against weevils in order to produce the first crop of alfalfa, and in many cases to protect the stand, is common knowledge and practice over most of Maryland and in much of the adjoining infested area. The nature, type, and extent of injury as well as descriptions of the various stages of the weevil have been reported by many authors. Only brief mention will be made of them in this report.

Egg-Laying Habits.—Observations by many workers on the biology of the alfalfa weevil in the Western States have been summarized by Essig & Michelbacher (1933). It is generally agreed that early in the spring many eggs are laid in dead alfalfa stems but later in the season most of them are deposited in growing stems.

This is true in Maryland. Weekly samplings begun in mid-March 1956 showed a general increase in the number of eggs found in dead stems until mid-April, when the first eggs were found in growing stems. Hatching soon reduced to zero the number of eggs found in dead stems, but the number found in growing stems continued to increase (table 1). Beginning on April 6, 99 egg clusters containing a total of 871 eggs, an average of 8.8 per cluster, were observed.

A similar sampling in the fall of 1956 showed a few eggs in dead stems, but most of them were placed at varying heights inside growing stems. Egg clusters were also observed in petioles of trifoliate leaves and on one terminal bud, but these locations are considered atypical. Of 285 egg clusters observed in early November, only 11 were laid in dead stems, 7 in growing plant parts other than the stem, and the remainder in growing stems. The total number of eggs in these clusters was 2,737, or an average of 9.6 eggs per cluster.

Undoubtedly many of the eggs found in dead stems in the spring were laid in growing stems the preceding fall, but some were probably laid in dead stems in the spring before growing stems were large enough.

In the Western States the act of oviposition begins with the female weevil cutting a tiny circular hole with her mouth parts. She then removes her mouth parts and inserts her ovipositor. After oviposition the hole is generally plugged either by the last egg inserted or by a secretion. No differences in this process have been observed in Maryland except that the plug frequently resembles "bits of epidermis chewed from the stem," or feces, as described in Delaware by Milliron & MacCreary (1955). Sweetman (1929) presented data indicating that low relative humidity retarded embryonic development, which may give a reason for the insertion of the eggs into stems and the sealing of the punctures.

In April 1953 an unusual type of plant injury was observed in a field in Baltimore County, Md., where oviposition was extremely heavy: there were as many as eight egg punctures per stem, which was the apparent cause of the death of many stems.

Incubation Period.—In Maryland the incubation period of alfalfa weevil eggs varies greatly with the time of the year. Milliron & MacCreary (1955) have observed that in Delaware eggs are able to survive the winter. Western workers disagree on this point. According to Titus (1913) fall-laid eggs are killed by low winter temperatures, but Snow (1925) and Reeves *et al.* (1916) indicate that fall-laid eggs do hatch the following spring. As pointed out by Essig & Michelbacher, these conflicts may be accounted for by differences in climatic conditions under which the investigations were conducted. Eggs do overwinter in Maryland.

Eggs deposited in the fall and throughout the winter in the insectary have been observed during the last three seasons. Most of the fall-laid eggs were deposited in November. In two of these years eggs laid in January failed to hatch and in the third no eggs were laid in February. The numbers laid in January and February were always very low, and they rarely were deposited between mid-January and mid-February.

A summary of insectary hatching records is presented in table 2. It can be seen that many eggs laid before November hatched in the fall. This was not true in 1954, when the limited number observed were all laid on the 29th of October. With this exception, the longest incubation periods were for November-laid eggs, and the period progressively shortened after that month. As warm weather returned eggs again hatched in a minimum observed time of 9 days.

A few observations on summer-laid eggs were made during the summer of 1956. A total of 13 eggs hatched in an average of 7.3 days, with an incubation range of 4 to 10

Table 1.—Spring egg laying by the alfalfa weevil as observed by examining alfalfa stems in five 1-square-foot samples, Beltsville, Md., 1956.

Date	Dead Stems		Growing Stems	
	Clusters	Eggs	Clusters	Eggs
March 23	7	—	0	0
30	3	—	0	0
April 6	9	63	0	0
13[a]	11	84	2	17
20	3	28	8	76
27	6	41	15	123
May 3	0	0	46	439

[a] First hatching observed.

[1] Accepted for publication June 26, 1957.

[2] R. B. Knapp, deceased, assisted with these studies during 1955.

Table 2.—**A summary of hatching records of alfalfa weevil eggs held at outdoor temperatures in an insectary, Beltsville, Md.**

Month Laid	Month Hatched	Number of Eggs Hatched	Incubation Period (Days)	
			Range	Average
1953–54				
October	November–January	572	34–123	88.7
November	March–April	1472	102–142	117.9
December	March–April	576	91–126	111.2
January	No hatch	—	—	—
February	April	18	51– 56	51.5
March	April	116	19– 31	24.1
April	April	13	9– 18	14.5
1954–55				
October	March–April	6	152–173	160.3
November	April	23	100–143	127.9
December	April	13	101–124	112.3
January	April	6	95– 98	96.3
February	No eggs deposited	—	—	—
March	April	12	26– 42	30.4
1955–56				
September	October	196	11– 31	17.9
October	October–April	129	8–168	32.5
November	February[a] and April	422	66–170	143.3
December	April	86	128–140	132.0
January	No hatch	—	—	—
February	April	6	61– 61	61.0
March	April–May	70	35– 67	48.1
April	April–May	1066	11– 39	17.3
May	May–June	374	9– 27	14.1

[a] Three larvae hatched, but all died in first instar.

days. The incubation period for all years varied from a minimum of 4 days for eggs laid in July 1956 to 173 days for eggs laid in late October of 1954.

LARVAL AND PUPAL STAGES.—In the Western States the newly hatched larva makes its way up the outside of the stem to the terminal growth of the alfalfa plant, is generally concealed there, and feeds on the bud during the first two instars. Later instars are found feeding on the older leaves. There is no doubt that this is the most destructive stage. No differences in larval behavior have been observed between the Western States and Maryland.

In Maryland eggs have been laid and hatched in every month of the year. The greatest number hatch in the early spring, and by far the greatest number of larvae are found in late April and in May. A very few larvae are found throughout the summer months and into early December, but none have been collected in the field during the winter. It is therefore assumed that larvae hatching in the late fall or possibly warm days during the winter do not survive in the field.

App (1954) states: "Larvae of all sizes were collected as late as December 8, indicating that at least a part of the population has more than one generation a year." Further observations on these larvae, a total of 235 collected from September 1 to December 8, 1953, showed that adults were reared only from those collected on or before October 23. Larvae collected as late as November 4 did pupate, and the pupae appeared viable until the first of March, but none produced adults. The lowest recorded temperature during that period was 6° F.

Early in November 1956 an attempt was made to rear newly hatched larvae. Some survived and fed for as long as 2 weeks, but died apparently as a result of low temperatures.

Essig & Michelbacher (1933) state that in the Western States "upon reaching maturity the larvae either crawl or drop to the ground where they spin a delicate net-like cocoon of white silken threads." In first reporting on the alfalfa weevil in the East, Poos & Bissell (1953) observed that many spun their cocoons and pupated on the host plant rather than dropping to the ground. Similar observations were made in the following years. This difference may be due to the generally higher relative humidity in the East. This explanation is based on the assumption that the habit of dropping to the ground prior to pupation in the West is an effort toward moisture conservation, which is not necessary in the more humid East.

Field observations were made on the occurrence of the first larva and pupa and first empty cocoon each spring for the period 1953–56. The averages dates, with extreme dates given in parenthesis, were April 9 (April 2 to 14), May 4 (April 22 to May 15), and May 14 (May 3 to 18), respectively. The average time between the first larva and the first pupa was 25 days, and between the first pupa and the first empty cocoon, 10 days.

SEASONAL HISTORY.—The adults feed vigorously upon leaves and stems of alfalfa plants immediately after their emergence in the late spring and early summer. Then most of them apparently leave the alfalfa fields and do not return until fall. Adult activity and larval abundance are greatest in April and May while the first crop of alfalfa is making its growth. During this time larvae cause severe injury in virtually every field in the infested areas of the State, and it is necessary to use insecticides in order to produce an acceptable crop. During mid-May it is common to take 3,000 to 5,000 larvae with 100 sweeps of a 15-inch net in untreated fields. This population peak is terminated by pupation and the cutting of the first hay crop. Some continuation of this population often extends into the second hay-cutting period. After that period their presence and feeding injury seldom attract notice.

In order to determine weevil activity during the remainder of the season, sweeps were taken in several fields at 7- to 10-day intervals from June to December. Samplings made for the 4 years are presented in figure 1. The counts from year to year are probably influenced by the number of untreated fields included in the sample. In all years the larvae were reduced to less than one per sweep early in July, and in 3 years there was a slight increase in mid-July or early August. From that time until October there was usually some activity of both adults and larvae, but it was very light until the adults again returned to the alfalfa fields. In October and November there was more adult activity and in some years a very slight increase in larval activity. It is doubtful if these late-season larvae mature to adults in Maryland. A second peak of adult activity, extending through December in 1956, reflects the unusually high temperatures during that period which followed a brief cold spell late in November.

After the late-fall peak the sweep samples again indicated little or no adult activity. However, this time it may be due to hibernation in the crowns of the plants, under the surface litter, and to some extent in the soil. To gain more information on this point, in July 1956 a series of observations were begun in a single field by a modification of the method described by Hamlin *et al.* (1949). Five 1-square-foot samples were taken at weekly intervals, and examined for adults, larvae, pupae, and eggs. In addition, once a month the soil in each sample was removed to a depth of approximately 1 inch and examined by washing on screen sieves.

Small numbers of eggs were found in the stems in mid-

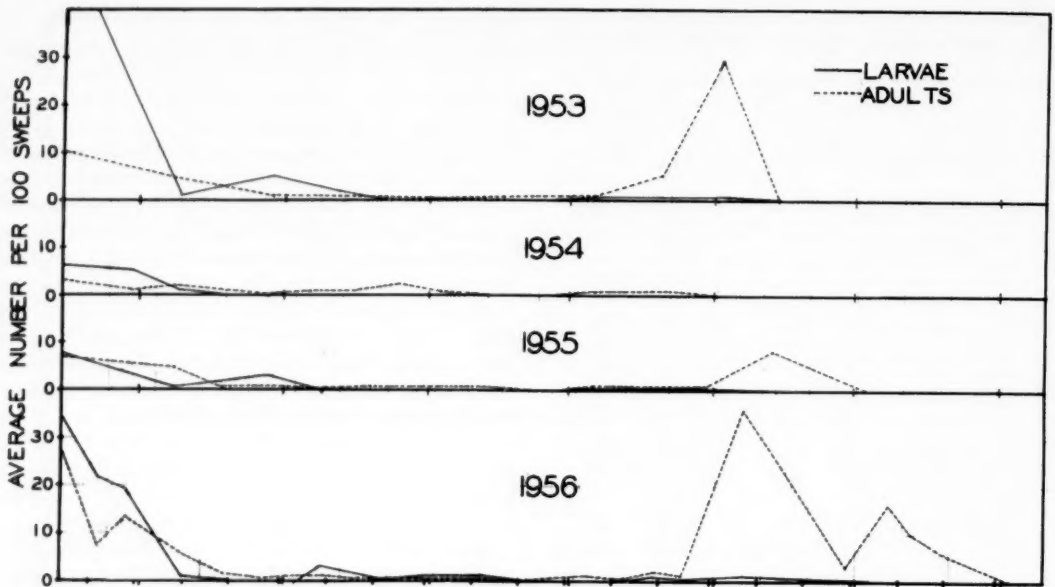

FIG. 1.—Summer and fall activity of the alfalfa weevil as observed by sweeping in alfalfa fields in Maryland. The 1953 observations were made in Harford County, and the number of fields and number of sweeps on each date varies. In the other years 100 sweeps were made in each of 10 fields in Prince Georges and Howard Counties.

July and early August. After August 28 eggs were not found again until October 17, when there was an average of 0.4 cluster per square foot. The number of egg clusters increased to a peak of 47.8 per square foot on December 17. This number is probably unusually high. The field in which the samples were taken was seeded in the fall of 1955, but had never been treated with insecticides and only one cutting of hay was taken from it during the 1956 season. Samples taken in January 1956 from several other fields in the area indicated only 2.6 egg clusters per square foot.

Of particular interest is the number of adults found. Only one adult was taken from the soil betwen July 17 and October 2, but nine adults were taken on the first of November, 34 on December 9, and five on January 29.

Each year since 1953 newly emerged weevils were placed in cages with alfalfa and held in the insectary at outdoor temperatures. Invariably there was no oviposition until late October or November. This was true whether the adults had emerged in June or September. This timing corresponds with the peak of fall egg laying as observed in the field. The pre-oviposition period varied from 57 days for adults emerging in September to 157 days for those emerging in June. A similar period of adult sexual immaturity has been reported by many workers, and is an important factor in the seasonal history of the weevil because it greatly reduces the possibility of more than one generation in a season.

In July 1956 a number of adults were reared from field-collected larvae in the insectary. One group of two females and four males were fed from the date of their emergence on July 11 until about August 1, but from that time until October 5 they were accidentally starved. On the latter date fresh food was supplied to these weevils and on November 5 eggs were deposited, an indication that little injury had occurred during the period without food. More work needs to be done on this point, but this observation tends to support the field observations that most adult weevils leave alfalfa fields during the summer.

Hamlin *et al.* (1949) reported a similar situation in Utah, where the new-generation adults moved from the fields to field borders and ditch banks during the summer, and Titus (1913) states: "We have found that a large number of the weevils in the summer flight leave the alfalfa fields, and since they are distributed in every direction, they will be fortunate if even a small percentage reach other fields." In Maryland no congregations have been observed in ditch banks or field borders, as in Utah. However, a great many adults apparently return to alfalfa fields in the fall. It is possible that some of them hibernate out of alfalfa fields and do not return until spring.

DISCUSSION.—Adults and eggs are capable of passing the winter in Maryland, but neither of these stages enters a truly dormant period. Fall-laid eggs generally do not hatch until spring; however, they occasionally hatch in the winter even when held at outdoor temperatures and readily hatch when incubated at higher temperatures. Adult behavior follows a similar pattern, in that they are inactive while held at low temperatures but will lay eggs on warm days during midwinter. Present evidence strongly indicates that larvae and pupae will not survive winter temperatures in Maryland.

Although adults emerging in June and held at outdoor temperatures did not lay eggs until late October or November, eggs and larvae have also been found during the summer months, with a slight increase in late July and early August. Essig & Michelbacher (1933) conclude that the eggs and larvae found during the summer months in the Western States are from females of the old generation. Milliron & MacCreary (1955) believe that there are two

generations in Delaware. It is possible that in Maryland some of the early-emerging adults, resulting from overwintered eggs, may be sexually mature by midsummer and responsible for the larvae found. All the newly emerged adults that have been observed were ones emerging in June or later. However, whether or not the summer larvae represent a new generation is purely an academic question, because their numbers are much too small for them to inflict economic damage or to be responsible for the adult populations that return to the fields in the fall and eventually give rise to the larval population of the following spring.

SUMMARY.—The alfalfa weevil (*Hypera postica* (Gyll.)) was first reported in Maryland in 1952. From 1953 to 1956 observations were made on its biology and seasonal history in this State.

Most of the eggs are laid in growing stems but a small number were found in dead stems. The maximum incubation period was 173 days for eggs laid in the fall and the minimum 4 days for eggs laid during the summer. The average first date of occurrence for larvae in the spring is April 9.

The alfalfa weevil apparently has one generation a year in Maryland. Egg deposition in the fall is interrupted by cold weather and resumes again in the spring. Spring-laid eggs hatch in about 2 weeks. The larvae feed and increase in size until early May, when pupation begins. Adults begin emerging shortly before the time of the first hay cutting. Most of these adults do not lay eggs until the following fall. There is some adult and larval activity in alfalfa throughout the summer, but it is not believed to represent a true second generation.

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On Laboratory Colonization of the Eye Gnat, *Hippelates collusor* Townsend[1,2]

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Hippelates eye gnats have long been extremely annoying pests in the Coachella Valley of California (Herms 1926, Hall 1932, Burgess 1935). They persistently fly about and alight upon the face and are a great nuisance to farmers and farm workers. These gnats may also be abundant in residential areas and in school yards. A gross correlation between abundance of eye gnats and incidence of an infectious eye disease, pink eye, common among children, strongly suggests that eye gnats are vectors of the causative agent (Herms 1926, Hall 1932, Burgess 1951, Tinkham 1953).

Current studies on the biology and control of *Hippelates* eye gnats were begun in 1956. Since it is desirable to have laboratory populations of mature and immature stages for biological studies, insecticidal screening tests, studies with attractants and other uses, efforts were directed toward finding an easy, workable mass-rearing technique under laboratory conditions.

The rearing technique described here was developed for the colonization of *Hippelates collusor* Townsend, the predominant pestiferous species in the Coachella Valley, with a high level of populations prevailing over a period of 8 months of the year. It is probable that the breeding procedure developed for *H. collusor* may be applicable to the breeding of the other species of eye gnats as well, but the possibility of certain specific requirements cannot be ruled out. Adults of *H. dorsalis* Loew and *H. robertsoni* Sabrosky feed readily on the diet developed for *H. collusor*, and most of the confined individuals lived beyond 8 days. Further studies are required, however, on the utility of this technique for rearing other *Hippelates* species.

With the use of the materials and procedures described below, *H. collusor* has been reared for eight generations in the laboratory. It is not uncommon, using a cage filled with a freshly prepared larval medium, to obtain an emergence of 5,000 to 6,000 gnats per cage when stocked with 300 to 400 male and female gnats. Certain aspects of the rearing technique are not final as yet, and studies

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[2] Paper No. 973, University of California Citrus Experiment Station, Riverside, California. Studies carried out under grant from the Coachella Valley Mosquito Abatement District, Riverside County. Accepted for publication June 27, 1957.

are under way toward improvement and simplication.

REARING CAGES AND DEVICE FOR WATERING LARVAL MEDIUM.—Cages constructed for the purpose of rearing *Hippelates* must provide: (1) sufficient ventilation of the upper part of the cage; (2) adequate light for adult activity; (3) space above the rearing medium that is adequate and yet sufficiently confining to keep the ovipositing adults near the surface of the medium; and (4) a practicable depth of the lower part of the cage where the breeding medium for the larvae is placed. Depths exceeding 5 or 6 inches have been found to result in poor aeration, unsatisfactory wetting, and putrefraction of the medium.

The rearing cages currently in use (fig. 1) are constructed of either pine or redwood and are 18 inches long, 12 inches wide, and 11 inches deep. The hinged upper portion of the cage, confining the adults, measures 5 inches in height, while the lower portion has a depth of 6 inches. A 40-mesh plastic screen $3\frac{1}{2} \times 13\frac{1}{2}$ inches is stapled on the front and back part of the upper compartment of each cage to provide ventilation and adequate light. Regular canning jar rings are fitted into each end of the upper unit. Quart jars may be fitted into these to facilitate removal or introduction of gnats. To furnish food and water; three 1-inch holes are drilled in the top of the cage. A 30-ml. dropping bottle containing water and a $\frac{1}{2}$-inch thick cotton wick is inserted into the center hole to furnish a constant source of water for the adult gnats. Absorbent dental rolls impregnated with food are held in 1-dram vials fitted into corks. These corks in turn are fitted into the two outside holes to insert the dental rolls into the cage.

A simple gravity and suction feed-water mechanism (fig. 1) has been developed for keeping the larval medium uniformly damp. The amount of water flow depends on the height of the water head in the water container and the moisture content of the vermiculite mixture. Watering need arises at first when the medium is 5 days old and then again when it is 2 weeks old, in order to provide enough moisture for the second generation larvae. A 1-gallon container can provide water to four cages at the same time by conducting the water flow through Tygon and Y glass tubings. Water is injected into the medium through a smallbore copper tube inserted in the middle of the cage.

LARVAL MEDIUM.—Under natural conditions *Hippelates* larvae are soil inhabitants. Their abundance and development depend on the moisture and organic-matter content of the sandy soils in which they live. On the basis of this information, alfalfa meal was mixed with various quantities of a coarse sand to yield 5, 10, and 20% organic matter. Approximately 2% of brewer's yeast was also added. These mixtures gave a low yield of gnats. Heavy weight, need for frequent watering, and crusting over of the surface layer were also drawbacks of these mixtures.

Seeking a more suitable base for preparation of a breeding medium, we compared clay loam, medium sand, Vermiculite 2, and Vermiculite 4 (California Zonolite Company, Los Angeles) as to their water absorption and retention capacities on a dry-weight basis under laboratory conditions. Vermiculite 2 and Vermiculite 4 absorbed about 20 times as much water as medium sand and clay loam. Under similar conditions the loss of water was considerably slower from vermiculite materials than from either sand or clay loam. Vermiculite 4 was selected because of its finer particle size and because of less shrinkage upon drying.

Alfalfa meal was added to the dry Vermiculite to make a 20% mixture. Brewer's yeast in the amount of 150 to 200 gm. was added per 6 pounds of the dry mixture. The materials were mixed thoroughly and wetted by the

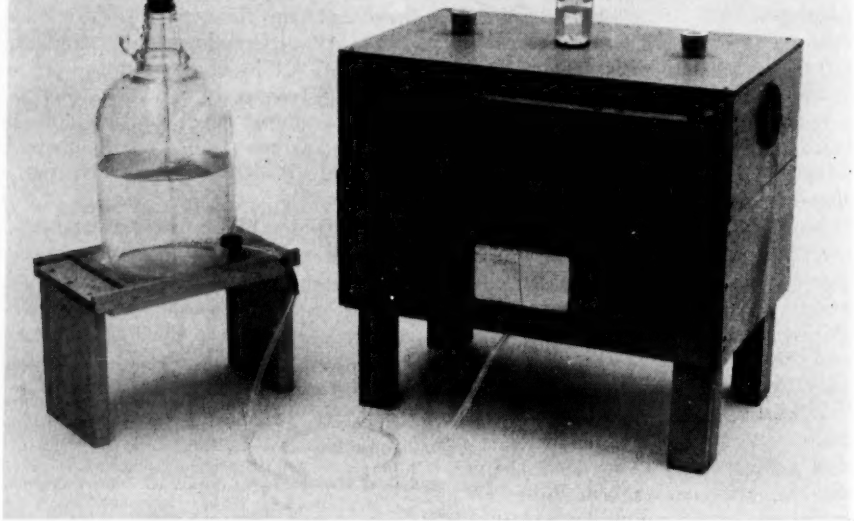

FIG. 1.—Rearing cage and watering device used in colonization of the eye gnat, *Hippelates collusor* Townsend.

addition of 2 gallons of water. The wet mixture was transferred to the rearing cage, and 300 to 400 gnats were added to each cage. The surface of the medium should be disturbed at an interval of 2 to 3 days in order to expose damp medium for oviposition.

The medium thus prepared is light in weight and produces no malodors. Following initial preparation, the temperature of the larval medium rises by about 25° F. above that of the room temperature, for about 3 days. This feature of the medium has the advantage of shortening the life span from egg to adult stage, from about 4 to 5 weeks to about 2 to 3 weeks. This is a desirable attribute of the technique, particularly in cases where large and frequent turnover of populations are needed.

Only two generations were completed in this mixture. The old mixture may be renewed by adding alfalfa meal and brewer's yeast. However, higher production resulted when freshly made medium was used.

Other species of flies may contaminate the stock from time to time. A fungus gnat, *Bradysia* sp.[3] (Lycoriidae), and a scavenger gnat, *Scatopse fucipes* Mg.[3] (Scatopsidae), became abundant in the rearing cages when sand, alfalfa meal and brewer's yeast mixtures were utilized as the larval medium. These flies dwindled to insignificant proportions as the vermiculite mixture was substituted.

In the vermiculite mixture another dipterous species, *Desmometopa singaporensis* Kert.[4] (Millichiidae), appeared in abundant numbers. This highly fecund species interfered with *Hippelates* production severely by completing one generation in 10 to 11 days. This species was eliminated by a combination treatment, fumigating the cages and discarding the medium from which these flies emerged.

Litter-breeding mites of the genus *Anoetus*[5] (Anoetidae) are currently appearing in the rearing cages. The nonfeeding deutonymphs (hypopi) of these mites attach to insect with their suckers for the purpose of transportation. Numbers of hypopi on *H. collusor* adults in the laboratory colony were noted to range from a few to several hundred, and in a few cases even up to a thousand individuals, on one gnat. A count indicated an average of 600 to 700 mites per gnat. The gnats when heavily infested are unable to feed or drink and their movement is greatly impaired.

To get a good emergence of mite-free gnats, it is necessary to treat the top 1 to 2 inches of the medium with a spray containing 1% of actual Dimite, using a 25% emulsifiable concentrate. The spray is applied at the rate of 50 ml. per cage. This treatment resulted in satisfactory control, and the newly emerged gnats were practically free of mites. A 40% Dimite wettable powder applied at the rate of 1-gram actual toxicant per cage, either as spray (50 ml. per cage) or dust, failed to give satisfactory control. Similarly, a spray of 2% actual Aramite, using 25% emulsifiable concentrate, applied at the rate of 50 ml. per cage, gave no appreciable reduction of mites on the emerging gnats. None of these materials seem to be toxic to the gnats when used at the indicated dosages.

For best results, the cages should be treated a short time after the start of emergence. Treatments applied too early did not ordinarily result in good control of the mites. Studies are under way to find a suitable and effective acaricide that can be added to the medium during mixing.

FEEDING OF ADULTS.—Hall (1932), during his studies on the eye gnats in the Coachella Valley, investigated the longevity of adult gnats under artificial conditions. They were fed with sugar-water solution, meat juices, fruit juices, and various types of decaying vegetable matter. None of the gnats survived beyond 7 days in the experimental cages. Some preliminary observations by the present workers under laboratory conditions (temperature 85 ± 2° F. and relative humidity 20 to 25% in subdued light) indicate that *H. collusor* has a preoviposition period of 6 to 8 days. The problem of maintaining gnats beyond their preoviposition period may have been the cause of negative results of previous colonization attempts.

Adult eye gnats will feed readily on honey, sugar, protein hydrolysate solutions alone or in combination, as well as on other nutritious substances such as fruit juices and blood. The rate of mortality, however, is relatively high when food is offered in solution. The abdomens of the gnats swell and many of them regurgitate copious amounts of the solutions that they have just fed upon. This creates a sticky environment in which the movement of the gnats is hampered. Microbial and fungal growths in the liquid media further add to the undesirability of such solutions or liquid diets, and fresh solutions have to be prepared frequently. It therefore becomes necessary to furnish the gnats with food in some other convenient form on which they will feed readily. This was accomplished by soaking absorbent dental rolls in the liquid diet and then drying them.

PREPARATION OF FOOD FOR THE ADULTS.—No information is available on the nutritional requirements of *Hippelates* adults. In developing this rearing procedure a diet was prepared that should contain ample supply of nutrients and essential substances, based on a general knowledge of insect nutrition. It is recognized that some of the materials used in the diet may be present in amounts in excess of requirements.

In the course of preliminary feeding experiments, it was found that addition of blood or blood albumin resulted in increased feeding by the adults. Moreover, this material might also furnish nutrients and elements required for egg development. Therefore, fluid blood was added to the other ingredients of the diet. To avoid mold growth on the food rolls during storage and through the duration of exposure in the cages, a small amount of sodium propionate was also made a constituent of the diet. All the materials used in the preparation of the solution for impregnation of dental rolls are shown in table 1.

A solution containing all the ingredients except cholesterol and corn oil was prepared in an 800-ml. beaker by weighing out the major components and then adding the indicated volumes of the various stock solutions. Several portions of water were added with periodical stirring, until all the ingredients went into solution or suspension. The final volume of the solution was then brought to 650 ml. with water.

A 20-foot length of ½-inch absorbent dental roll was cut into 3-inch lengths. Each one of these lengths, in turn, was cut lengthwise except at one end, so that the uncut

[3] Identified by Alan Stone, Entomology Research Division, U. S. Department of Agriculture, Washington, D. C.

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[5] Identified by I. M. Newell, University of California, Riverside.

Table 1.—Diet provided adults of the eye gnat, *Hippelates collusor*, in laboratory rearing procedure.[a]

Materials	Amount (Gm.)	Materials	Per Cent Solution	Volume (Ml.)
A. Honey	60.0	C. KH_2PO_4	0.10	
		K_2SO_4	.320	
MRT protein hydrolysate	22.5	$MgSO_4$	.040	30
		NaCl	.092	
Partially hydrolyzed yeast	7.5			
		D. Fe Citrate	0.052	
Staley's sauce base 2	15.0	$MnSO_4\ 4H_2O$	.036	
		Cu acetate	.001	9
Human whole fluid blood	30.0	Zn acetate $2H_2O$	.024	
Sodium propionate	0.5	E. H_3BO_3	0.0125	
		NH_4 Molybdate	.0350	6
B. Cholesterol	1.0 in ether			
Corn oil	4.0 in ether	F. $CaCl_2$	0.092	2
		G. Choline Cl	0.25	4

[a] All ingredients except corn oil and cholesterol were brought into aqueous solution. The final volume was made to 650 ml., and 20 ft. of ½-inch thick dental roll (cut into small lengths) were soaked in the diet solution. Ethereal solution of corn oil and cholesterol was then atomized over the wet rolls, and these, in turn, were dried.

end could be held in vials fitted into the cages. These lengths of dental roll were soaked in the diet solution until no further absorption took place. They were then placed in a tray and sprayed over both sides with the ether solution of corn oil and cholesterol, using about 10 ml. of this solution over the 20-foot length. The dental rolls were completely dried in an air-blast oven at 95 to 100° F. This took about 8 to 10 hours. The impregnated and dried rolls were placed in an air-tight plastic container and stored in a refrigerator.

On exposure in the cages, the rolls absorbed moisture and became damp and soft within a few minutes. One or two diet rolls were placed in each cage, the number depending on the number of gnats present. It was found that two rolls furnished food for a period of 7 to 8 days for 2,000 to 3,000 gnats. It was more desirable to change the diet rolls alternately every 3 or 4 days.

The diet prepared for *H. collusor* as described above seems to be complete and palatable. The gnats fed on the diet rolls in great numbers, and the majority of the adults provided with this food lived for a period of 6 to 8 days (85 ±2° F. and relative humidity 20 to 25%) beyond the start of oviposition.

Studies are under way to simplify this diet and compare the effects of various ingredients on the longevity of adults and extent of oviposition.

SUMMARY.—The eye gnat *Hippelates collusor* Townsend is by far the most abundant species found in the Coachella Valley of California. A practicable mass rearing technique in the laboratory has been worked out for this insect. The larval medium consists of Vermiculite No. 4, alfalfa meal and brewer's yeast. The mixture is wetted with water and placed in specially constructed wooden cages. The medium in the cages is then kept damp uniformly by a simple watering device.

A palatable, complete diet in dry form has been developed for the adults. This diet is easy to handle and does not need frequent preparation. Once prepared, the diet can be stored for months in a refrigerator. Adult eye gnats provided with this food lived 6 to 8 days beyond their preoviposition period.

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Developments in Chemical Control of the Spotted Alfalfa Aphid in California, 1955–56[1]

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Since its establishment in New Mexico, probably in 1953, the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), has spread rapidly over an extensive part of the North American Continent. By January, 1957, this aphid could be found from coast to coast and from central Wisconsin on the north, deep into Mexico on the south. After analysis it is not surprising that this aphid should spread over such a vast area in a relatively short period. Nor does it now appear unusual that it has become one of the major pests of alfalfa in the United States, in contrast to its minor importance in the Mediterranean region and Middle East areas to which it is native (Harpaz 1955, van den Bosch 1957).

In the Old World, certain alfalfa strains show a resistance to the spotted alfalfa aphid (Stanford 1956). Undoubtedly, over the centuries the aphid has been selective in weeding out susceptible plants, and peasant farmers have unknowingly taken seed from the hardier ones for cultivation. Also in its native home, the aphid is attacked by a number of predators and parasites which further act to hold the density of the species at a sub-economic level. When the aphid arrived in the United States, it found an unlimited food supply in the millions of acres of susceptible alfalfa varieties grown across the United States. Moreover, it arrived without its parasites and thus without significant checks; the species was relatively free to multiply to a tremendous density. As each offspring constitutes a new focal point of establishment, the aphid spread rapidly with the wind and by vehicle across the central part of the North American Continent.

In California, the first damaging population occurred in the Imperial Valley in June, 1954 (Dickson *et al.* 1955). This insect can now be found throughout the major alfalfa-producing areas of the state and is the most important insect pest ever to attack alfalfa in California. The damage to alfalfa caused by the feeding of the aphid has been described by Reynolds & Anderson (1955) and by Parker *et al.* (1956). It is now widely known, particularly in the southwestern part of the United States, where severe losses have occurred for the past 3 years.

Prior to the establishment of the aphid in California, alfalfa growers generally produced this crop with only an occasional treatment for insect pests. With the aphid to combat, however, the frequency of chemical treatments in alfalfa fields has increased markedly. Since alfalfa is a basic argicultural crop in California, with about 1.2 million acres planted to alfalfa hay and seed in 1956, the sudden appearance of this insect has had an adverse effect on the economic status of many alfalfa growers. This is especially true on farms where alfalfa production comprises a sole source of income, for in some areas this aphid has turned alfalfa production into a nonprofitable enterprise through its attack on the crop over the entire growing season.

It is estimated that in 1956 over 85% of the California alfalfa acreage was at some time subjected to attack by this aphid. In southern California, where one-fourth of the state's alfalfa is grown, potentially damaging infestations continued from one cutting to another over a good portion of the 1956 growing season. In some areas potentially damaging populations built up and were controlled with chemicals, built up again and were controlled, and on occasion again increased to damaging numbers, all within a 30- to 40-day cutting cycle. Where aphid attack is continuous, not only have production costs increased, but in many fields there has been a reduction in yield, quality, and stand of the alfalfa.

In addition to these economic factors, a developing resistance by the aphid to parathion in localized areas of southern California threatens to complicate the problem further (Stern & Reynolds 1957). In some cases, growers failed to obtain satisfactory control when using ½ pound or more than twice the recommended dosage of parathion applied with a ground sprayer. Through further selection of resistant aphids, it is possible that parathion may become totally useless as a control measure; first, by failing to give adequate control at a low dosage; and second, from a cost factor, where a high dosage of parathion is necessary to give satisfactory results. Until resistant alfalfa varieties are released and the introduced parasite species *Praon palitans* Mues., *Trioxys utilis* Mues., and *Aphelinus semiflavus* How. (van den Bosch 1956) become widely established in California, insecticides will continue to play a prominent role in the control of this pest. Even after resistant alfalfa varieties have come into widespread use and the parasites have reached maximum effectiveness, chemicals may still be required at times for control of this pest.

During 1955 and 1956, a large number of field experiments were conducted to evaluate the effect of new compounds for aphid control. It is not practical to present the data from every test; therefore, only a few of the most significant tests are reported in detail. These tests showed that two new compounds, Trithion (originally Stauffer R-1303; Stauffer Chemical Co.) and Phosdrin (originally Shell OS-2046; Shell Chemical Corp.), were particularly promising for spotted alfalfa aphid control on alfalfa. These materials also appear to possess chemical characteristics which should permit their registration for use on alfalfa hay or seed, or both. Consequently, a major portion of the field work reported herein was directed toward an extensive evaluation of these compounds.

EXPERIMENTAL METHODS AND RESULTS.—All the chemicals studied in these field tests were applied as sprays. The method of evaluating each compound was the same in all tests, based on cutting single alfalfa stems just above ground level and counting the living apterous aphids on them. In all experiments except one, each treatment was randomized and replicated four times. Twenty-five alfalfa stems were counted in each of the four repli-

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cates to give a total of 100 stems per treatment. In one test, only two replicates were made, and two separate counts of 25 stems each were taken in widely separated areas within each replicate, or a total of 100 stems per treatment. Effectiveness of a treatment was based on its reduction of aphids as compared with the check.

Test 1.—On November 2, 1955, several materials were applied as sprays on alfalfa in the Imperial Valley. All treatments were applied with a ground sprayer equipped with No. 4 hollow cone nozzles set at 18-inch intervals for broadcast spraying. The sprayer was operated at a pressure of 60 p.s.i. Each replicate was 27 feet wide and 150 feet long. The alfalfa was about 15 inches tall. The treatments were evaluated 9, 15, and 21 days after application. Results are summarized in table 1.

In this experiment, Trithion was applied at two dosage levels, these being 14 and 7 ounces per acre. Nine days after application the 7-ounce dosage of Trithion gave a 99% reduction in the aphid population, which was comparable to that of parathion, methyl parathion, and malathion applied at 5.6, 5.8, and 13.0 ounces per acre, respectively. Twenty-one days after application, all spray materials appeared to be holding the aphid in check. However, aphid activity in the Imperial Valley is normally at a low ebb during November, and the apparent residual toxicity from a number of the materials is thus misleading. During periods of heavy alate aphid flights, parathion, methyl parathion, and malathion do not give residual toxicities lasting through a 21-day period when applied at the concentration used in this test. Guthion applied at 5 ounces per acre gave satisfactory aphid control, although it was not quite so effective as the other spray materials applied in this test.

In two field tests for aphid control on alfalfa prior to this experiment, Trithion gave similar results. In the first of these two tests, Trithion was applied at 9.5 ounces per acre and gave initial control comparable to that of demeton applied at 4.1 ounces and malathion applied at 11.7 ounces per acre. Trithion at this dosage gave indications of a residual toxicity similar to that of demeton and superior to that of malathion. In the second test, Trithion applied at 6.4 ounces per acre gave control comparable to that of parathion applied at 3.2 ounces per acre.

Test 2.—On July 24, 1956, spray materials were applied on alfalfa in the Antelope Valley, a large alfalfa-producing area at the western edge of the Mojave Desert. Treatments were applied as in Test 1. Each replicate was 60 feet wide and 100 feet long. The alfalfa was about 16 inches tall. The treatments were evaluated 1, 3, and 7 days after application. Results are summarized in table 2.

Trithion at 13.5 and 7.3 ounces per acre gave better than 99% control 24 hours after treatment at both dosages. Parathion used as a standard comparison material and applied at 2.9 ounces per acre also gave better than 99% control 24 hours after treatment. In this test both concentrations of Trithion maintained very effective control through the seventh day after application, further indicating a long residual toxicity, even when applied at approximately ½ pound per acre. Seven days after application, control had decreased slightly in the plots treated with parathion. The increasing population was almost entirely first-instar nymphs from dispersing alate aphids, showing a moderate residual toxicity when parathion was applied at a low dosage.

Two spray mixtures of Chipman R-6199 were applied. In one mixture 4 ounces of the technical chemical were mixed with 15 gallons of water, and in the second, the same amount of chemical plus 1¼ quarts of glycerol were mixed with 15 gallons of water. Glycerol was added to the second spray mixture to delay recrystallization of the compound. With delayed evaporation it was hoped that additional chemical would be absorbed into the plant, giving a localized systemic effect as apparently occurs when the material is used in high-volume sprays. However, neither preparation gave satisfactory control. The summarized data in table 2 indicate a delayed toxic action in both preparations of the spray mixture. Field observations revealed that predators which moved into the plots after application were probably responsible for the substantial reduction of the aphid populations. These effects of predators are indicated by the population trend in the untreated plots. Without the presence of natural enemies, the number of aphids in the untreated plots would have increased greatly between the 3- and 7-day evaluation period; but as the data show, this did not occur.

In another field experiment conducted in the early summer of 1955, the compound *O,O*-diethyl *S*-2-(diethylamino) ethyl phosphorothiolate (R-7707), the base of Chipman R-6199, was tested for aphid control. It failed to give satisfactory control when used at 1.9 ounces per acre. In the same test, Chipman R-6199 applied at 2 ounces per acre gave less than 50% control.

Table 1.—Effectiveness of various insecticide sprays applied by ground equipment on November 2, 1955, for control of the spotted alfalfa aphid on alfalfa in the Imperial Valley, California.

Material	Gallons/ Acre	Ounces Toxicant/ Acre	Days Between Treatment and Sampling: 9 — No. Apterous Aphids/ 100 Stems	9 — Per Cent Reduction From Check	15 — No. Apterous Aphids/ 100 Stems	15 — Per Cent Reduction From Check	21 — No. Apterous Aphids/ 100 Stems	21 — Per Cent Reduction From Check
Trithion	14.0	14.0	5	99	4	99	45	99
Trithion	14.0	7.0	15	99	18	99	73	98
Parathion	13.5	5.6	12	99	4	99	46	99
Methyl parathion	14.8	5.8	22	99	18	99	59	98
Malathion	12.8	13.0	35	99	66	98	162	96
Guthion	14.0	5.0	208	97	156	95	370	90
Check	—	—	7,332	—	3,232	—	3,749	—

Table 2.—**Effectiveness of various insecticide sprays applied by ground equipment or by airplane for control of the spotted alfalfa aphid on alfalfa in the Antelope Valley, California.**

Material	Gallons/ Acre	Ounces Toxicant/ Acre	Days Between Treatment and Sampling: 1 — No. Apterous Aphids/100 Stems	1 — Per Cent Reduction from Check	3 — No. Apterous Aphids/100 Stems	3 — Per Cent Reduction from Check	7 — No. Apterous Aphids/100 Stems	7 — Per Cent Reduction from Check
Test 2—Application by ground equipment July 24, 1956								
Parathion	14.5	2.9	3	99	56	99	292	96
Trithion	12.7	13.5	3	99	2	99	26	99
Trithion	13.6	7.3	10	99	13	99	55	99
Chipman R-6199	13.6	3.6	1,055	70	1,368	79	399	95
Chipman R-6199[a]	12.1	3.2	1,317	63	1,110	83	214	97
Check	—	—	3,542	—	6,491	—	7,946	—
Test 3—Application by airplane July 30, 1956								
Trithion	3	8	22	99	154	97	226	—
Trithion	3	4	105	95	284	95	1,092	—
Check[b]	—	—	2,099	—	5,096	—	[b]	—

[a] Glycerol (1¼ quarts added to 15 gallons of water in spray mixture).
[b] Check plots treated 5 days after experiment began.

Test 3.—On July 30, 1956, spray materials were applied on alfalfa in the Antelope Valley by airplane, using approximately 3 gallons of spray mixture per acre. Each replicate was 160 feet wide and 1,240 feet long. The treatments were evaluated 1, 3, and 7 days after application. The data are summarized in table 2.

Trithion, an organic phosphate insecticide with a low vapor pressure, has little if any systemic action. Since the spotted alfalfa aphid does not move about the plant while feeding, a good coverage with this material is essential for maximum effectiveness. This experiment was conducted on alfalfa being grown for seed. The field was in 20 to 30% bloom; the plants were largely mature, with heavy lateral branches. Strong westerly winds, common in the Antelope Valley, had lodged many of the mature plants. In those areas of thick growth, the lodged plants formed a tangled mat covering the lower portions of the plants. This thick mat prevented the spray droplets from filtering through to the lower parts of the plants. Twenty-four hours after treatment, 8 ounces of Trithion had given 99% control while 4 ounces gave 95% control. In both treatments a number of adults and older nymphs still remained on the plants. The great majority of the aphids surviving treatment were found on the lower leaves in those areas where the alfalfa had lodged to form a protective covering. Three days after application, control had decreased slightly in both treatments, the majority of the aphids being first-instar nymphs produced by the surviving adults. At this time the population in the large untreated plots had increased to potentially damaging numbers and they were treated with 4 ounces of demeton per acre. Seven days after beginning the test, 8 ounces of Trithion still held the aphids in check. Where 4 ounces were applied, the population was increasing quite rapidly, especially in the areas where the alfalfa had lodged. Ten days after the test started re-treatment of the plots by the grower eliminated further evaluation of the treatments. It would appear from this experiment that 4 ounces of Trithion applied by aircraft on heavily matted alfalfa will not effectively control this insect or a spray application of 3 gallons per acre will not give sufficient coverage for maximum effectiveness of Trithion, or the combination of the two may have been responsible for the mediocre aphid control.

Test 4.—On August 21, 1956, spray materials were applied on alfalfa near Mojave in the northwestern part of the Mojave Desert. All treatments were applied with a ground sprayer equipped with No. 4 hollow cone nozzles arranged 18 inches apart for broadcast spraying. The sprayer was operated at a pressure of 60 p.s.i. Each replicate was 80 feet wide and 100 feet long. The alfalfa was about 12 inches tall. The treatments were evaluated 1, 3, and 8 days after application. Results are summarized in table 3.

In this test Phosdrin and Trithion were both applied at two concentrations: Phosdrin at 3.3 and 1.4 ounces per acre, and Trithion at 8.9 and 4.5 ounces per acre. Twenty-four hours after application, both dosages of these materials gave better than 99% control, which was comparable to that of parathion used at 3.4 ounces per acre. Furthermore, field observations showed that in both treatments with Phosdrin the majority of the aphid population was knocked off the plants within 30 minutes after application. In addition, the the few lady beetles which were in these plots were also killed soon after the Phosdrin was applied. All treatments of Phosdrin, Trithion, and parathion continued to maintain satisfactory control through the 8-day evaluation period. However, there was apparently a very low alate aphid flight throughout the area during the testing period, for later tests with Phosdrin showed that where rapid reinvasion of the plots occurred, residual toxicity did not exceed a 24-hour period. The compound *O,O*-dimethyl *S*-2-(propynyl) phosphorodithioate (CP-11327) was applied at approximately 9 ounces per acre and failed to give satisfactory control.

Eight days after the sprays were applied, the field was irrigated by means of a sprinkling system. The alfalfa in the untreated plots and in the plots treated with *O,O*-dimethyl *S*-(2-propynyl) phosphorodithioate (CP-11327) was stunted and partially defoliated by the aphids. This

Table 3.—Effectiveness of various insecticide sprays applied by ground equipment for control of the spotted alfalfa aphid on alfalfa in test plots in various parts of California.

Material	Gallons/ Acre	Ounces Toxicant/ Acre	Days Between Treatment and Sampling: 1 — No. Apterous Aphids/100 Stems	1 — Per Cent Reduction from Check	3 — No. Apterous Aphids/100 Stems	3 — Per Cent Reduction from Check	8 — No. Apterous Aphids/100 Stems	8 — Per Cent Reduction from Check
Test 4—Mojave Desert plots; sprayed August 21, 1956								
Phosdrin	15.7	3.3	0	100	43	99	207	98
Phosdrin	14.3	1.4	0	100	67	99	236	99
Trithion	16.3	8.9	0	100	11	99	97	99
Trithion	16.8	4.5	59	99	32	99	115	99
Parathion	16.1	3.4	3	99	29	99	231	98
CP-11327[a]	16.1	8.9	1,803	86	6,704	73	5,526	56
Check	—	—	12,666	—	24,581	—	12,633	—
Test 5—Owens Valley plots; sprayed October 9, 1956								
Parathion	12.0	2.4	7	99	1	99	125	97
Phosdrin	12.2	0.6	2	99	179	90	1,038	75
Phosdrin	11.8	0.3	212	83	755	58	5,105	—
Trithion	10.0	2.9	386	69	87	95	405	90
Check	—	—	1,251	—	1,774	—	4,198	—

[a] *O,O*-dimethyl *S*-(2-propynyl) phosphorodithioate (Monsanto Chemical Co.).

sprinkler irrigation washed a good number of the aphids from these denuded stems. Since the sampling method would not account for the aphids washed from the plant, the average number of aphids per stem recorded for these two treatments on the eighth day after application does not represent an accurate measure of the population present immediately prior to the sprinkler irrigation.

It should be noted that this was not the first test in which Phosdrin was used. In two previous tests, one using a ground sprayer and the other using an airplane, the vapors from the Phosdrin eliminated the aphids from the untreated plots. In the first test, 16-ounce and 8-ounce applications of Phosdrin were applied on small replicated plots with a ground sprayer, and in the second test 1-ounce and 3-ounce applications were applied on large replicated blocks with an airplane. Both tests were conducted in very hot weather when there was very little air movement in the treated area for at least 12 hours. In each case the aphids in the untreated plots were eliminated by the Phosdrin vapors.

Test 5.—On October 9, 1956, spray materials were applied on alfalfa near Big Pine in the Owens Valley at the eastern base of the Sierra Nevada Mountains. All treatments were applied in the same manner as those in Test 4. The plots were 50 feet wide and varied in length from 510 to 1,240 feet in the triangular-shaped field. The alfalfa was about 15 inches tall. Treatments in this test were replicated only two times. However, two separate counts of 25 stems each were taken in each replicate to give a total of 100 stems per treatment. Samples were taken 1, 3, and 8 days after the plots were treated. Beginning on the day of treatment through the eighth day after application, the daytime temperatures were moderate, but night temperatures dropped slightly below freezing. Results are summarized in table 3.

Phosdrin was applied at two dosage levels: 0.6 and 0.3 ounce per acre. Where 0.6 ounce of Phosdrin was applied, control was satisfactory 24 hours after application. Where 0.3 ounce was applied, the control appeared to be satisfactory 24 hours after treatment. However, field observations showed this was a sublethal concentration to a certain percentage of the population. One day after treatment nearly all the aphids were knocked off the plants, but a large number were crawling about near the base of the plants. Three days after application many of the surviving aphids had found their way back onto the alfalfa. This low dosage of Phosdrin appeared to be toxic to predators, which may account for the unusual increase in population following treatment. One day after application 0.6 ounce of Phosdrin per acre gave better than 99% control. Two days later, control decreased to 90% and then to 75% 8 days after application. During the experiment a heavy flight of alate aphids was occurring in the testing area. These alates were readily observed in the Phosdrin plots. A number of first-instar nymphs located somewhat randomly on the plants indicated the very short residual toxicity of Phosdrin.

Trithion was applied at 2.9 ounces per acre and gave 69% control 1 day after application. The majority of the aphids remaining at this time were mature adults. Control increased to 95% 3 days after application and decreased slightly 8 days after the material was applied. Parathion applied at 2.4 ounces per acre gave very effective control during the testing period.

SUMMARY.—The spotted alfalfa aphid, *Therioaphis maculata* (Buckton), probably became established in New Mexico in 1953. By January, 1957, it could be found within a wide belt across the central part of the North American Continent. Damage to alfalfa has been severe for the past 3 years in the southwestern part of the United States. In some areas this aphid has turned alfalfa production into a virtually nonprofitable enterprise through its attack on the crop over the entire growing season.

Field tests conducted during 1955 and 1956 revealed two new compounds, Phosdrin and Trithion, which were very effective for spotted alfalfa aphid control. Trithion

was applied in sprays with dosages varying between 2.9 and 14.0 ounces per acre. Control was better than 99% 24 hours after application when 4.5 to 14.0 ounces per acre were applied with a ground sprayer. When applied at 2.9 ounces per acre with a ground sprayer, reduction of aphids over untreated plots reached 95% 3 days after application. For consistently good results, at least 4 ounces per acre applied with a ground sprayer may be necessary. Trithion, an organic phosphate insecticide with a low vapor pressure, has little if any systemic action; thus a good coverage is essential for maximum effectiveness. The material has a long residual toxicity on the aphid when applied at 8 ounces per acre.

Phosdrin was applied in sprays with dosages varying between 0.3 and 3.3 ounces per acre. Three-tenths of an ounce per acre is a sublethal dosage, with many aphids recovering after being knocked off the plants. When applied at 0.6 ounce and above, control is effective soon after application. Phosdrin has a very high vapor pressure and when applied during high temperatures with little air movement, its vapors are very effective in controlling the aphid. During periods of heavy alate aphid flight, control with this material does not exceed a 24-hour period. Preliminary observations indicated that Phosdrin was toxic to many beneficial insects.

Trithion would appear to have an advantage over Phosdrin in that it has greater residual toxicity. However, the short residual life of Phosdrin may prove advantageous when aphid control is necessary close to cutting.

Parathion, methyl parathion, and malathion gave satisfactory aphid control when applied at 2.4, 5.8 and 13.0 ounces per acre, respectively. Guthion applied at 5.0 ounces per acre gave satisfactory control, although it was not quite so effective as the three materials just mentioned. Control was not satisfactory when Chipman R-6199 was applied at 3.2 or 3.6 ounces per acre or when *O,O*-diethyl *S*-2-(diethylamino) ethyl phosphorothiolate (R-7707) was applied at 1.9 ounces per acre. *O,O*-dimethyl *S*-(2-propynyl) phosphorodithioate (CP-11327) also gave mediocre control when applied at approximately 9 ounces per acre.

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Chromatographic Separation and Identification of Some Alkaloid Metabolites of Nicotine in Certain Insects[1]

F. E. GUTHRIE, R. L. RINGLER, and T. G. BOWERY[2]

Despite the fact that a number of insects attack tobacco, practically nothing is known of the physiological mechanism that enables them to survive on the relatively large quantities of nicotine they must ingest. A thorough study of the relationship between such insects and this alkaloid should not only give us a better understanding of this host relationship, but it should also present new concepts on the resistance of insects to insecticides.

No work appears to have been published on the metabolism of nicotine in insects. Metcalf (1955) has recently summarized the literature dealing with the toxicity of nicotine and related compounds toward insects. Richardson *et al.* (1934) and Glover & Richardson (1936) showed that nicotine could penetrate directly through the cuticle as well as through the spiracles. They found that penetration was quite rapid, and large amounts of "nicotine" could be recovered from the cuticle with smaller amounts from the muscles, fat body, digestive tract, and nerve cord. The analytical procedure used was not specific for nicotine so the "nicotine" probably included nicotine and other closely related alkaloids, including metabolites. In a later paper, Richardson (1945) speculated that the degree of susceptibility to nicotine might be due to differences in rate of penetration, slow pentration permitting detoxification.

In mammals, it is well known that man is able to acquire a considerable tolerance to nicotine. Wahl (1919) has shown that confirmed tobacco smokers are unaffected by amounts of the alkaloid (8 mgs.) which would cause marked symptoms in non-smokers. The chief site of detoxification are the liver with lesser amounts found in the lung, kidney, brain, and muscle (Werle & Meyer 1950). The principal metabolite was found to be nicotyrine, and other partially identified metabolites were also found (Werle *et al.* 1950). Nicotine and nicotyrine are rapidly excreted by the rat. Forty per cent was excreted in 3 hours, 85% in 6 hours, and nearly all in 16 hours (Ganz *et al.* 1951).

It is the purpose of this paper to present preliminary

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results of a study of the metabolism of nicotine in insects. The studies reported here were conducted on topically or orally treated insects which were sacrificed at various time intervals and the metabolites extracted, separated by paper chromatography, and identified, at least in part.

METHODS.—Fifty micrograms of nicotine (99%) were applied to the dorsum of the first thoracic segment of the adult male German cockroach, *Blattella germanica* (L.). With the American cockroach, *Periplaneta americana* (L.), 300 micrograms were applied per adult female cockroach in a similar manner. The southern armyworm, *Prodenia eridania* (Cram.), was treated orally by offering thin wafers of potatoes which had been dipped in a 0.5% solution of nicotine in chloroform. Fifty German cockroaches, 20 American cockroaches, and 35 southern armyworms were used in each test jar. German cockroaches were analyzed after 5, 10, 56, and 120 hours, American cockroaches at 10, 56, and 120 hours, and southern armyworms at 8, 29, 54, and 96 hours. Although the cockroaches showed marked symptoms of nicotine poisoning initially, the majority recovered within 24 hours.

After each of the exposure periods the insects were removed and the holding jars washed three times with 15 ml. of a saturated solution of CaO. The solution was then filtered and the filtrate extracted four times with 15 ml. portions of chloroform. The aqueous layer was discarded. The external portions of the insects were washed twice with 25 ml. portions of chloroform and held for treatment as described below. The insects were then ground in a mortar and pestle with 5 to 10 ml. of a saturated solution of CaO. The macerated alkaline slurry was allowed to stand for 1 hour. Ten ml. of chloroform was then added and the mixture filtered. Four 10-ml. portions of chloroform were used to wash the macerated tissues. The chloroform extracts of the jar wash and those of the external and internal insect extracts were then washed four times with 15 ml. of 2 N HCl, and the organic layer was discarded. The acid solutions were then made alkaline to litmus paper with NaOH, and the alkaloids re-extracted with chloroform. This step was found necessary to remove some of the interfering materials. The solution of alkaloids in chloroform was then evaporated to 1 ml. and stored in a refrigerator until ready for the chromatographic procedure.

The chloroform extracts were separated by ascending paper chromatography. The methods and solvents used were similar to those of Tso & Jeffrey (1953) using t-amyl alcohol or the solvent system of Frankenburg *et al.* (1955) using benzene-methanol-acetate buffer. One hundred μl. spots were applied to Whatman No. 1 filter paper at 5- to 7-cm. intervals. The edges of the sheet were stapled together to form a cylinder and the sheet allowed to equilibrate with the aqueous phase for at least 4 hours. The organic phase was then added and the chromatograms

Table 1.—Comparison of the Rf values (×100) of suspected metabolites of nicotine in insects in three solvent systems with certain known alkaloids.

TREATMENT	SOLVENTS			COLORS SHOWN FOR SPOTS FOUND WITH TERTIARY AMYL ALCOHOL
	T-amyl Alcohol Acetate Buffer[a]	Benzene-Methanol-Acetate Buffer[b]	80% Acetone[c]	
German cockroaches (50 roaches treated topically with 50 micrograms/roach for each of four time intervals)	1. 90±3 2. 85±4[d] 3. 37±5	1. 66±5[d] 2. 31±5 3. 15	1. 80 2. 74[d]	Yellow-orange Yellow-orange
American cockroach (20 roaches treated topically with 200 micrograms per roach for each of three time intervals)	1. 95 2. 90±4[d] 3. 40±5	1. 64±4[d] 2. 31±5 3. 15	1. 75	Light yellow Yellow-orange to pink Yellow
Southern armyworm (Fed potatoes dipped in 0.5% nicotine solution to 35 worms for each of four time intervals)	1. 94±2 2. 88±4[d] 3. 79±5 4. 40±5 5. 17±4	1. 96±1 2. 94±1 3. 85±4 4. 71±6 5. 53±5 6. 25±2 7. 15±2 8. 7±4 9. 0.0	1. 85 2. 77 3. 60	Orange-yellow Orange-pink Orange pink Yellow Orange
Potato section dipped in nicotine solution	1. 95±1 2. 91±1 3. 45±7	1. 97 2. 95 3. 89 4. 40		Orange-pink Yellow Yellow
Nicotine	42±6	37±9	82	Dull yellow
Nicotyrine	95±2	97±2	96	Pink
Metanicotine	28±6	0.0	—	Pink
Myosime	92	93	—	Bright yellow
N-methyl nicotinamide	91±2	19±4	78	Yellow
Cotinine	89±2	67±5	76	Orange
Nor-nicotyrine	96	—	—	Violet

[a] Average of 10 or more runs.
[b] Average of 4 or more runs.
[c] Average of 2 runs.
[d] Main metabolite.

allowed to develop for 16 to 18 hours with t-amyl alcohol, 10 to 12 hours with the benzene-methanol-acetate, and 7 hours with 80% acetone. All chromatograms were developed at 85° F. in an air-conditioned room. The chromatograms were removed at the desired time intervals, air dried, and sprayed lightly with a 1% solution of p-aminobenzoic acid in alcohol. The chromatograms were then placed in a chamber containing vapors of cyanogen bromide for 30 minutes. The yellow to red spots were lightly circled and the Rf values calculated. The colors reached maximum intensity in 20 to 30 minutes and quickly faded. A visual quantitative estimate of the metabolites was not considered accurate enough to be reported.

Certain of the compounds were also subjected to ultraviolet absorption spectroscopy. The unknown or known fractions were applied to a sheet in an unbroken line and developed as previously described. After drying, a side strip was treated with the color reagents. The location of bands in the main part of the paper was inferred from that found in the colored strips. The bands were then cut out and eluted with 0.25 N HCl to a total volume of 8 to 10 ml. A portion of paper taken from a blank sheet developed similarly was also leached to use as a blank. The ultraviolet absorption spectra were determined on a Warren Spectracord. The peaks were generally found to be sharp and narrow indicating relatively pure compounds except with fractions from the German cockroach in which cases the peaks were somewhat poorly defined.

The extracts from the German and American cockroaches were compared with cotinine using a Perkin-Elmer, Model 21, infrared spectrophotometer. Estimated 100- to 300-microgram quantities were used with the K Br pellet technique.

RESULTS AND DISCUSSION.—The Rf values of the chromatograms are shown in table 1, and the unknown spots resolved by the three solvents are listed in order of their decreasing Rf value in each solvent. Figure 1 shows

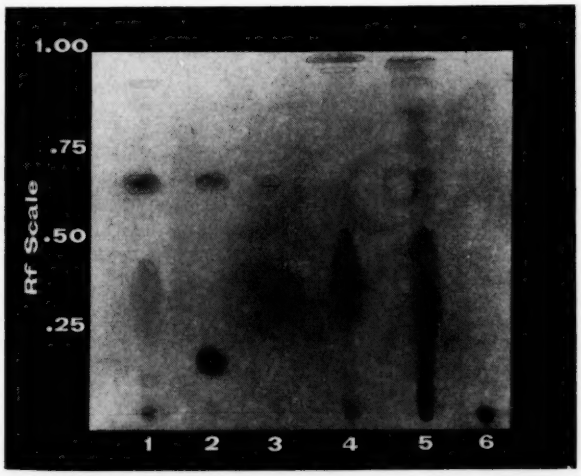

FIG. 1.—Typical chromatogram developed with benzene-methanol-pH 5.6 acetate buffer mixtures: (1) American cockroach, (2) Cotinine (above) and N-methyl nicotinamide (below), (3) German cockroach, (4) Nicotyrine (highest), myosime (next highest) and nicotine (below), (5) Southern armyworm, (6) Metanicotine.

Table 2.—Comparison of the maximum ultraviolet absorption spectra of some suspected metabolites of nicotine with certain known alkaloids.[a]

TREATMENT	Rf VALUE (×100) OF BAND[b]	MAXIMUM PEAK
German cockroach	90	1. 233
	85	2. 261.5, 255.5
American cockroach	90	261.5
Southern armyworm	94	1. 259.5
	88	2. 260.5
Nicotine	42	259
Nicotyrine	95	302, 241
Myosime	92	263
N-methyl nicotinamide	91	262.5
Cotinine	89	261.5

[a] Measured in 0.25 N HCL.
[b] Developed in t-amyl alcohol.

a typical chromatogram. In control experiments neither macerated tissues nor excretory products of untreated insects gave colored spots.

Experiments using 80% acetone as the chromatographic solvent were made with only a selected few of the various fractions Therefore, it is possible that more metabolites might have become evident had this solvent been used in all cases.

The peaks of the ultraviolet absorption spectra in aqueous acid solution are shown in table 2, and figure 2 shows a typical set of such curves. The data shown are for the main metabolite(s) found for each insect. No attempt has yet been made to identify the less important metabolites which were especially numerous in the southern armyworm.

Cotinine was found to be essentially non-toxic to the German cockroach; an injected dosage of 200 micrograms (in acetone) per male cockroach caused no appreciable mortality.

The results shown in tables 1 and 2 present evidence which suggests that cotinine is the main metabolite of nicotine degradation in the American cockroach. The Rf values in three solvent systems were quite similar, and the maximum peaks of the ultraviolet absorption were identical. The metabolites from the German cockroach had Rf values similar to those from the American cockroach in the three solvent systems mentioned and had certain similarities on the ultraviolet absorption spectrogram. The peaks were rather broad, however they do not exclude the possibility of cotinine being present. An attempt was made to obtain an infrared spectrum on the main metabolites from each of the cockroaches using the limited amount of materials available. Apparently a trace of silicone stopcock grease was inadvertently introduced. Since the silicone bands interfere with several of the cotinine bands, interpretation could not be conclusive but did present some additional information. The spectra for cotinine and the metabolite in the American cockroach appear identical insofar as the silicone bands do not interfere. The spectrum for the German cockroach appears to be very slightly different in the 7- to 9-micron region. Since the chromatograms eluted for the absorption spectra were all developed with the same solvent system, it is possible that the main metabolite eluted for the German cock-

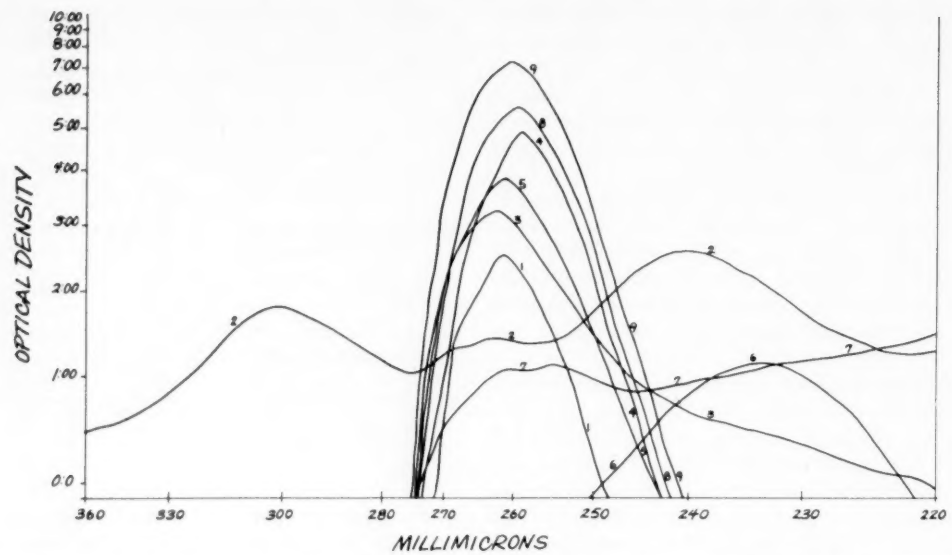

FIG. 2.—Ultraviolet absorption spectra of selected alkaloids and metabolites from insects developed in t-amyl alcohol-acetate buffer: (1) Cotinine, (2) Nicotyrine, (3) N-methyl nicotinamide, (4) Nicotine, (5) American cockroach metabolite, (6) German cockroach metabolite 1, (7) German cockroach metabolite 2, (8) Southern armyworm metabolite 1, and (9) Southern armyworm metabolite 2.

roach did not represent a single substance. A compound closely related to cotinine or cotinine plus an impurity might result in the observed spectral behavior of the main metabolite band from the German cockroach. A better technique is needed to clarify this phase of the work.

The results with the southern armyworm were quite different from those with the cockroaches. A larger number of spots were detected with the various solvent systems. The main metabolite had Rf values slightly different from those of the cockroaches. It also had slightly different ultraviolet absorption characteristics and was not identical with any of the known alkaloids tested. Certain of the other metabolites had Rf values similar to one or more of the known alkaloids, however sufficient material for spectroscopic examination was not available. It should be noted (table 1) that part of the nicotine on the potatoes was broken down in the control experiment (*i.e.*, without the presence of southern armyworms). The amount was small; however, it cannot be completely discounted as a source of part of the breakdown for this particular insect. Whether this was due to an enzymatic action in the potatoes or due to autoxidation of nicotine (as has been found by Frankenburg & Vaitekunas 1957) has not yet been determined.

The metabolites were not excreted in sufficient quantities to detect in the German cockroach until 10 hours. They were detected in the external and internal extracts at the 5-hour interval. The occurrence of a metabolite in the external cockroach wash was quite consistent. It is possible that the metabolites were either stored in the cuticle or considerable breakdown occurred in this region and when the external part of the cockroach was washed, the metabolites were withdrawn. However, this explanation is not entirely satisfactory, and more work is needed on this point. In the American cockroach the metabolites were detected in the excretory products in about 10 hours. As in the German cockroach, considerable quantities were consistently detected in the external cockroach wash. In the southern armyworm the metabolites were detected in the excretory products within 5 hours.

After the alkaline solutions used for extraction of the original tissues or products had been extracted with chloroform they were tested for the presence of alkaloids with silicotungstic acid with negative results. However, this should not preclude the possibility of metabolites which would not react with this reagent. The same is true of the cyanogen bromide test. This test would be positive only if at least one of the N-containing rings was still intact.

Future work will include further identification of the unknown metabolites, attempts to make quantitative recoveries of the alkaloids present in the tissues, studies on the rate of penetration of nicotine, and site of action of the detoxification of nicotine.

CONCLUSIONS.—In the American cockroach cotinine was found to be the main metabolite, appearing in the excretory products within 10 hours. Cotinine was identified by having similar Rf values in three solvent systems and an ultraviolet absorption spectrum identical to the known compounds. In the German cockroach the main metabolite had Rf values similar to those of the American cockroach in the three solvent systems, but the absorption spectra were different in certain respects. At least nine metabolites were found in the southern armyworm, and none of these were positively identified. Cotinine was found to be relatively non-toxic to the German cockroach.

SUMMARY.—The metabolism of nicotine in topically treated German and American cockroaches and orally treated southern armyworms was studied. After extraction, the metabolites were separated by an ascending paper chromatographic technique and partially identified by comparative Rf values and ultraviolet absorption spectra. Cotinine was found to be the main metabolite in the American cockroach, and a compound thought to be

quite similar to cotinine was found in the German cockroach. A number of metabolites, as yet unidentified, were found in the southern armyworm.

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The Interaction of Piperonyl Butoxide with Malathion and Five Analogs Applied Topically to Male House Flies[1]

GEORGE W. WARE and C. C. ROAN[2]

The commonly known synergists of pyrethrum, including piperonyl butoxide, piperonyl cyclonene, sesamin, MGK-264, and n-propyl isome also synergize the synthetic pyrethroids, allethrin and cyclethrin (Schmidt 1955). Eddy *et al.* (1954) observed that several pyrethrum synergists increased initial kill and residual effectiveness of certain phosphorus compounds against the body louse. Hoffman *et al.* (1954) evaluated seven phosphorus compounds with 19 synergists against DDT-resistant house flies. The synergists were effective with all of the phosphorus compounds except malathion.

Brief reports of antagonism were made by Turner & Saunders (1947), Perry & Hoskins (1950) and Sakai *et al.* (1951). Rai *et al.* (1956) found that piperonyl butoxide was antagonistic with malathion to susceptible and DDT-resistant house flies. Craig (1956) using topical applications, found that piperonyl butoxide was synergistic in combination with malathion to the German cockroach, *Blattella germanica* (L.), and antagonistic to the Madera roach, *Leucopheae maderae* (F.).

Toxicity studies of parathion and its derivatives on bees and flies by Metcalf & March (1949) indicated that with the basic parathion structure phosphoryl-group compounds were more active than those with thiophosphoryl-groups, and methyl and ethyl esters were more active than higher homologues. Hoffman *et al.* (1954) believed that the chemical structure of the phosphorus compounds was important since some of the synergists were more effective with certain phosphorus compounds than with others. It is believed from this work and from a rather thorough search of the literature that the structure of malathion and its analogs has a direct influence on their relative toxicities to house flies as well as on their activity with the methylenedioxyphenyl-containing synergists, especially piperonyl butoxide.

It appears from the review of literature that the actions of the commonly used synergists are not understood. Little agreement is found in the theories of synergism, and the small agreement seems to be on how the synergists do not act, rather than how they act. Antagonism on the other hand is even less understood and far less investigated. It appears probable that the biological action of piperonyl butoxide is consistent and that the ultimate response of synergism or antagonism is a function of the chemistry and stereochemistry of the toxicant and the biochemistry of the experimental organisms.

MATERIALS AND METHODS.—Technical grade piperonyl butoxide[3] was used in all LD_{50} determinations of malathion. Piperonyl butoxide, 100%, was used in the synergism and antagonism investigations using analogs of malathion. The malathion was 99.6% pure. Compound 4389, which came from a pilot plant batch of the American Cyanamid Co., was estimated to have a purity of 80%. The calculated and observed phosphorus and

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[3] Piperonyl butoxide, technical grade and 100%, were supplied by the Fairfield Chemical Division, Food Machinery and Chemical Corporation, Baltimore, Md.

Table 1.—Phosphorus and sulfur content of malathion analogs.[a]

Compound Number	Calculated		Found	
	Phosphorus	Sulfur	Phosphorus	Sulfur
3975	8.64	17.899	8.3	17.4
4018	9.34	19.41	9.27	19.1
4075	10.25	21.21	10.2	21.4
4128	8.64	17.89	7.4	17.0

[a] Data provided by Clark (1957). The malathion and its five analogs were supplied by the American Cyanamid Co., Stamford, Conn.

sulfur contents of the four remaining malathion analogs are given in table 1.

Topical applications of the toxicants alone and with piperonyl butoxide in acetone solutions were made using a microapplicator described by Roan & Maeda (1953). The $\frac{1}{4}$ cc. tuberculin syringe was calibrated to deliver 1.08 microliters of the acetone solution with each stroke of the microapplicator.

Male house flies (*Musca domestica* L.) of a DDT-susceptible strain (KUN) served as the test insects, and were reared under a modified Chemical Specialties Manufacturers Association system. The flies were sexed under CO_2 anesthesia on the third day after emergence and placed in a constant temperature chamber at 74 ± 2° F and room humidity, to be treated on the fourth day. They were fed exclusively on canned condensed milk and water, 1:1, after sexing and after topical treatment. This mixture was held on cellucotton in 1 ounce paper cups.

Holding and recovery containers were wide-mouth, pint Mason jars covered with a double layer of fresh cheesecloth held secure by rubber bands.

Fresh weighings of the toxicant and piperonyl butoxide were made daily and all dilutions were made from these. The dilutions were prepared with glass-distilled acetone using volumetric pipettes and held in cork-stoppered, 12-ml. glass shell vials.

Range-finding experiments to establish the proper dosage concentrations for LD_{50} determinations were made. Selected were four dosages that would give mortalities ranging rom 20 to 80% when applied to the mesosterna of the flies. Once the range was found test replications were begun.

For each replication four concentrations of the compound and four of the compound with piperonyl butoxide (1:10 by weight) were made shortly before topical applications were to begin. Each concentration was used to treat 100 flies, four jars of 25 each, so that for each

Table 2.—Chemical structures of malathion and five analogs, their toxicities alone and with piperonyl butoxide to male house flies.[a]

Cyanamid Compound	Structure	LD_{50} of Compound Alone	LD_{50} of CPD+P.B. Ratio (1:10 by Weight)	$\frac{LD_{50}CPD+P.B}{LD_{50}CPD}$
4018	$(C_2H_5O)_2PSCHCOOCH_3$ $\quad\quad\quad

replication 400 flies were treated with toxicant and 400 with toxicant and piperonyl butoxide. Applications were made beginning with the lowest concentration followed by acetone rinsing and drying of the syringe between concentrations.

At the end of 24 hours, mortality counts were taken. In these determinations a dead fly was one that showed no movement of the proboscis or legs.

LC_{50}s were obtained by plotting the mortalities on log-probit analysis sheets and eye-fitting the dosage mortality curve. The LC_{50}s were converted to LD_{50}s by use of mean fly weight and volume per stroke of the microapplicator.

Checks consisted of 25 untreated flies held in the temperature chamber with the treated flies.

RESULTS.—The results are summarized in table 2. Shown are the chemical structures of malathion, its five analogs and their relative toxicities. The LD_{50}s of the compounds alone and with piperonyl butoxide (1:10, by weight) and indications of antagonism or synergism are given.

With the compounds synergized the order of synergism is increased with a decrease in toxicity. With the exception of the position of compound 4075 the order of antagonism also increased with a decrease in toxicity.

Compounds 4018 and 3975 are homologous and the toxicity of the ethyl alkoxy homolog is enhanced by the presence of piperonyl butoxide.

DISCUSSION.—With malathion and the two homologous compounds, 4075 and 4128, the data suggest that an increase in the length of the alkoxy group from methyl to propyl increases the degree of antagonism with piperonyl butoxide.

Compound 4389 is synergized while malathion is antagonized by piperonyl butoxide. In this instance it seems that with this type of chemical structure the only difference between synergized and antagonized activity is the presence of the phosphoryl versus the thiophosphoryl group.

Compounds 4018 and 3975 are synergized while malathion, 4075 and 4128 are antagonized by piperonyl butoxide. The difference between these structures is the O,O-diethyl group attached to the phosphorus of 4018 and 3975, and the O,O-dimethyl group attached to the phosphorus of malathion, 4075 and 4128. This comparison suggests that the O,O-dimethyl group results in antagonism while the O,O-diethyl group results in synergism. A step further would be to predict the activity with piperonyl butoxide of a compound whose structure would be similar to 4018 and 3975 but having propyl alkoxy groups instead of the methyl or ethyl. If the hypothesis were true that with an increase in the alkoxy group length the relative toxicity would be reduced, an increase in synergism would be expected. This hypothesis is based on four observations: (1) an increase in synergism from 4018 with piperonyl butoxide to 3975 with piperonyl butoxide; (2) a decrease in relative toxicity from 4018 to 3975; (3) an increase in antagonism from 4075 to malathion to 4128; and (4) the difference between antagonized and synergized activity being the O,O-dimethyl or O,O-diethyl group attached to the phosphorus, or replacement of the thiophosphoryl with a phosphoryl group, as in 4389.

There is a possibility that these organo-phosphates may have varying degrees of solubility in piperonyl butoxide, which could account in some degree for their differences in activity with piperonyl butoxide. This aspect of the problem was not investigated.

The possibility of piperonyl butoxide impeding or enhancing absorption through the insect body wall of the toxicant has been investigated and will be presented in a later paper.

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SCIENTIFIC NOTES

Control of Onion Thrips in the Winter Garden Area of Texas, 1957[1]

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Experiments were continued in 1956 to evaluate new materials and to determine which chlorinated hydrocarbon insecticides have become ineffective in the control of the onion thrips, *Thrips tabaci* Lind. The possible tolerance of this insect to any of the phosphate materials was also an objective of these studies.

Tolerance of the onion thrips to some chlorinated hydrocarbons had been demonstrated at the Winter Garden Experiment Station (1955, 1956, 1957) and at the Lower Rio Grande Valley Experiment Station (1956).

PROCEDURE.—Two separate organic insecticide evaluation experiments were conducted on onions on a large farm northeast of Crystal City. The plots were 25 feet long and six rows wide. The plants were spaced 4 inches apart; two rows were planted on each plant-bed. The middle two rows of each plot were used for population records. Sprays were applied in both experiments with a three-gallon garden sprayer under 20 pounds pressure at the rate of 21 gallons per acre.

Records were taken by counting the onion thrips on the basal 4 inches of 10 consecutive plants selected at random in the middle rows of each plot, at intervals of 1, 4 and 7 days following application. The percentage reduction in infestation was based on the thrips population in the untreated check.

RESULTS.—The results of the two tests are shown in table 1.

Test 1.—Phosdrin, malathion, parathion and methyl parathion were the materials which gave excellent control the first day after application. The other materials were not satisfactory at that time. All of the materials tested remained effective for 4 days after application; treatments with parathion, malathion, Guthion, methyl parathion and Dow ET-15 (sampled as M-560) gave the greatest reduction in the thrips population. Both Dow ET-15 and Dow ET-57 (sampled as M-729) were noticeably slow in action but resulted in a fair reduction in population 4 days after application. A 0.75-inch rain fell on March 3 and 4, the third and fourth day after the insecticides were applied. This is probably a significant factor in the low reduction in population the seventh day after the materials were applied.

Test 2.—Chlorthion, and combinations of malathion plus dieldrin, malathion plus Perthane, and parathion plus dieldrin were the only materials resulting in effective reduction in thrips population 1 day after application in the second test. The combination of parathion plus dieldrin and Chlorthion alone were the only materials effective 4 days after application. Parathion plus dieldrin showed good control and Chlorthion, fair control, 7 days after application.

DISCUSSION.—Phosphate materials, at recommended dosages or the dosages used in these experiments, continued to give the most effective control of the onion thrips. Chlorinated hydrocarbons used in these tests were all noticeably ineffective in reducing thrips infestations. Malathion alone and parathion alone or both in combination with dieldrin, remain effective insecticides for the control of this pest. Malathion gave less control than parathion when each was combined with dieldrin. But, malathion in combination was used at half the rate at which it was applied when used alone; whereas, parathion was applied at the same strength alone and in combination. BHC plus DDT was very ineffective in this test.

Table 1.—Control of the onion thrips with organic sprays in the Winter Garden area in 1957.

POUND OF TOXICANT PER ACRE IN 21 GALLONS OF WATER	DAYS AFTER APPLICATION		
	1	4	7
	Per Cent Reduction		
Test 1:			
0.25 parathion	79.8	95.3	38.7
1.25 malathion	83.5	90.1	26.9
0.25 methyl parathion	73.0	85.7	18.8
0.5 Diazinon	62.0	72.8	16.5
0.25 Phosdrin	85.0	75.6	16.8
0.5 Guthion	43.4	88.9	53.2
0.5 Dow ET-15[a]	33.8	82.6	38.6
0.5 Dow ET-57[b]	56.1	74.2	18.7
Test 2:			
0.25 parathion plus 0..38 dieldrin	72.1	85.2	83.5
0.625 malathion plus 0.38 dieldrin	74.5	52.2	50.8
0.625 malathion plus 0.5 heptachlor	63.8	42.8	8.9
0.625 malathion plus 2.0 Perthane	72.9	64.6	50.9
0.3 BHC plus 0.5 DDT	42.0	13.0	15.6
0.5 dieldrin	27.4	12.8	0.0
0.5 heptachlor	33.4	30.3	31.1
2.0 Perthane	50.1	41.2	41.4
0.5 Chlorthion	76.1	81.1	63.1
0.5 Trithion	38.7	63.8	56.6

[a] Sampled as Dow M-560.
[b] Sampled as Dow M-729; also called Korlan.

Summary.—Two spray experiments were conducted in the Winter Garden area in 1957 for the control of *Thrips tabaci* on onions. One and 4 days after application, control of this insect was obtained by treatments with parathion, malathion, methyl parathion, Phosdrin and Chlorthion. The combination of parathion plus dieldrin was effective for 7 days after application. None of the chlorinated hydrocarbon insecticides controlled thrips when used alone. Resistance by the onion thrips to the combination of BHC plus DDT is indicated by the poor control recorded in these tests.

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[1] Accepted for publication June 5, 1957.

Evaluation of Synergized Pyrethrins on Various Strains of House Flies[1]

W. N. BRUCE[2]

A number of resistant strains of house flies (*Musca domestica* L.) were treated topically by means of a micro-syringe to determine the relative effectiveness of several synergized pyrethrins solutions. Test solutions were formulated on a weight per volume basis in acetone. Female flies were anesthetized with carbon dioxide to facilitate treatment. The treated flies were held at 80 degrees F. for a 24-hour mortality count. Mortality and dos-

[1] Accepted for publication October 3, 1957.
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Table 1.—LD-50 values in microliters of insecticidal solutions per adult female house fly of different strains.

Synergized Pyrethrins Solutions[a]	Fly Strain								
	CSMA-B	DDT-I	Lab. I-S	CSMA-A	Field I	Field II	Multi I	Malathion I	Malathion II
A	0.608	0.525	0.500	0.617			0.550		
B	1.025	1.100	1.000	0.950			1.710		
C	0.617	0.584	0.442	0.582			0.575		
D	0.573	0.567	0.335	0.550			0.400		
E	0.500	0.566	0.333	0.483	0.550	0.592	0.633		
F	0.500	0.575	0.433	0.470	0.583	0.600	0.683		
G	1.190	0.833	0.742	0.980	1.440	1.520	1.870		
H	0.487	0.475	0.325	0.453	0.466	0.517	0.633		
I	0.400				0.433	0.456		0.450	0.532
J	0.500				0.582	0.621		0.633	0.730

[a] Code:
A—0.1334% MGK-264, 0.080% piperonyl butoxide, 0.0167% pyrethrins.
B—0.50% MGK-264, 0.025% pyrethrins.
C—0.167% piperonyl butoxide, 0.0167% pyrethrins.
D—0.167% sesoxane, 0.0167% pyrethrins.
E—0.0267% pyrethrins, 0.053% piperonyl butoxide, 0.089% MGK-264.
F—0.134% piperonyl butoxide, 0.0267% pyrethrins.
G—0.5% MGK-264, 0.0267% pyrethrins.
H—0.134% sesoxane, 0.0267% pyrethrins.
I—2.0% MGK concentrate 933 in acetone (1.5% pyrethrins, 5.0% MGK-264, and 3.0% piperonyl butoxide).
J—2.0% Fairfield Chemical Division Roach Spray Concentrate in acetone (1.49% pyrethrins and 7.48% piperonyl butoxide).

age figures were plotted on logarithmic probability graph paper to compute the LD-50 values.

The strains of flies treated were as follows:

CSMA[3]-B	Laboratory susceptible
DDT-1	Highly DDT-tolerant
Lab I-S	Laboratory susceptible inbred strain
CSMA-A	Laboratory susceptible
Field I	1955 multitolerant field-collected strain
Field II	1955 multitolerant field-collected strain
Multi I	Laboratory multitolerant strain
Malathion I	1956 malathion and chlorinated hydrocarbon-tolerant field-collected strain.
Malathion II	1956 malathion and chlorinated hydrocarbon-tolerant field-collected strain.

Spray materials A, B, C, and D were used on one generation of flies and E, F, G, H, I, and J on another generation. Consequently, comparisons should be limited to data obtained within each generation of flies. Vigor variance between broods probably makes the comparison of synergized pyrethrins on different generations unreliable.

The LD-50 values shown in table 1 reveal a substantial difference among strains in their susceptibility to synergized pyrethrins.

Of interest was the high toxicity to house flies of the combination of piperonyl butoxide and MGK-264 with pyrethrins. MGK-264 with pyrethrins was found to have relatively poor insecticidal action on house flies. The combination of MGK-264 and piperonyl butoxide appears to offer a real economic advantage in producing synergized pyrethrins sprays equal in effectiveness to those synergized by piperonyl butoxide. To further verify these results, two commercial spray concentrates of approximately equal pyrethrins content were tested at 2% concentration in acetone. The pyrethrins spray containing the combination of MGK-264 and piperonyl butoxide was consistently better on the various strains of flies than the one containing only piperonyl butoxide as the synergist.

The reason for the advantageous mixture of synergists may well be a combination of two different modes of synergism.

The best single synergist tested was sesoxane, which was superior to the others at both the 5 to 1 and 10 to 1 ratios with pyrethrins.

[3] Chemical Specialties Manufacturers Association.

A Method for Rearing Leaf-Mining Agromyzidae[1]

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There is very little published information on methods of rearing leaf-mining Agromyzidae. Inchbald (1881) probably published the first method of rearing the adults from larval-infested leaves. In the method he described, no provision was made for maintaining the leaves in a turgid condition; therefore, many larvae undoubtedly perished in the desiccated foliage. Frost (1924) also made no effort to keep the leaves in a fresh state. Frick (1951) found it necessary to keep the leaves turgid for a period of 4 or 5 days to allow the larvae to reach maturity. This was accomplished by rolling the petioles of several leaves together in a damp cotton strip, thus forming a firm plug which was inserted into a water-filled vial. The vial was inverted in a pint jar to avoid the loss of the larvae by drowning in the damp cotton as they emerged from their mines.

Evidently, no attempt was made to induce oviposition by the adults under controlled conditions by either Inchbald, Frost, or Frick. McGregor (1914) confined adults of *Liriomyza pusilla* (Meig.) with seedling cotton plants in an unsuccessful attempt to induce oviposition. Claassen (1918) also failed in an attempt to induce mating and oviposition when he confined the adults of *Agromyza laterella* Zett. with iris plants. Webster & Parks (1913) succeeded in inducing the adults to oviposit in confinement. They also observed that confined flies fed readily on sugar water.

METHODS AND MATERIALS.—To furnish the nucleus of the colony that was needed for the rearing process, larval-infested leaves were gathered from plants in the field. The larvae were allowed to pupate and the resulting pupae were collected and confined.

Upon emergence of the flies, actual rearing of the colony began. The adults were confined with seedling snap bean or tomato plants in cylinders made of cellulose acetate and 6-inch embroidery hoops, the tops of which were covered with nylon stocking fabric (fig. 1). These cylinders were made by wrapping one end of a 16-inch-wide piece of acetate around the inner hoop and securing the outer hoop over it. Thus, the acetate was

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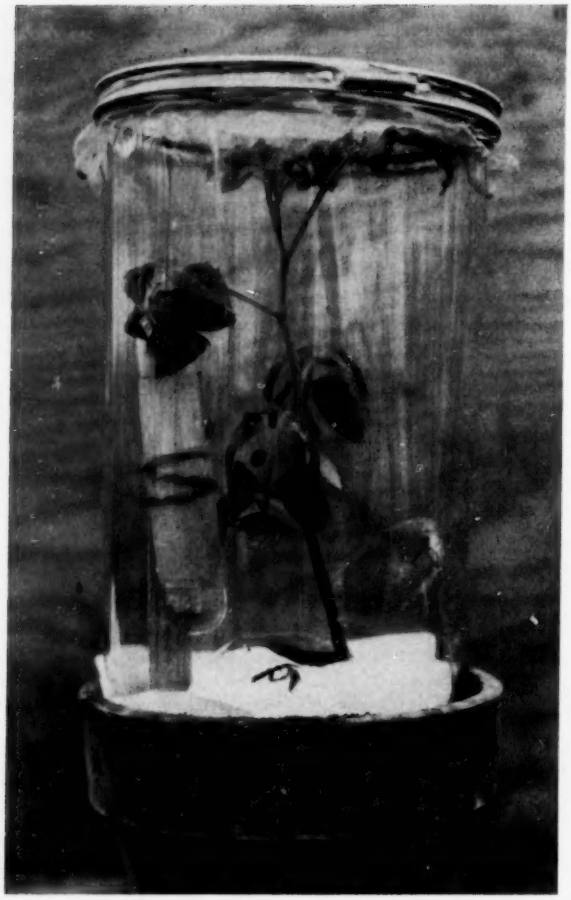

FIG. 1.—Cellulose acetate cylinder used to confine flies with the plants.

sandwiched between the hoops forming a rigid support for securing the nylon fabric in place with rubber bands. Cellophane tape was used to seal the seams of the cylinder.

An integral and most essential component of the rearing equipment was the artificial feeder. It consisted of a vial 1 inch in diameter into which two folded and rolled sheets of paper toweling were inserted. The tube was filled with a 20% solution of sugar water, which was absorbed by the paper toweling. This furnished a relatively wet surface upon which the flies could feed.

Seedling plants were grown in the greenhouse for use in rearing leaf miners. These were first seeded in beds or flats and transplanted to standard 6-inch pots after they had made sufficient growth. Flies were confined to the pots by acetate cylinders for a period of 2 or 3 days, depending on the number of flies in each cylinder. They were then changed to another plant to stay for approximately the same period of time.

After removal of the ovipositing flies, the plants were examined periodically in order to determine the progress of the larvae in the leaves. Approximately 1 day before the larvae reached maturity, the infested leaves were removed from the plants. The petioles of these leaves were inserted into a receptacle containing a solution of liquid plant food. This was done to allow the natural maturation of the larvae in the leaves. To prevent loss of larvae from drowning as they emerged from the leaves to pupate, the mouth of the container was covered with an index card held in place by rubber bands. Small holes were punched in the cover to allow access to the solution by the petioles of the leaves. The receptacle containing the infested leaves was then placed in a pupation chamber (fig. 2). The chamber consisted of a quart ice cream carton, the bottom of which was replaced by a disk of ¼-inch-mesh hardware cloth. The circular rim of the carton top was attached to the bottom of the chamber. The addition of this rim facilitated the temporary attachment of a ½-pint ice cream carton into which mature larvae dropped and pupated. This chamber functioned very effectively in the collection of pupae.

Periodically, the ½-pint cartons containing the pupae were removed, covered by a piece of nylon stocking fabric, and placed in a constant temperature box, maintained at 80° F., until the flies emerged.

The use of carbon dioxide as an anesthetic to facilitate the handling of the adults was unsatisfactory.

The success of this rearing method was dependent on the artificial feeding of the adults with sugar water to supplement the diet obtained from the plant. A simple test was conducted to determine the effect of sugar-water supplement on adult longevity. A group of 10 flies was confined with a plant plus a source of sugar water and an equal number of flies was confined with a plant alone. In both instances the test was replicated four times.

FIG. 2.—Pupation chamber used to confine larval-infested leaves.

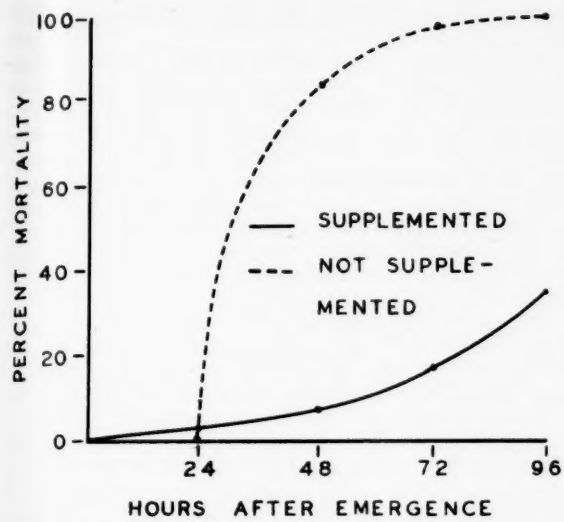

FIG. 3.—Mortality relationship between flies fed a diet supplemented with sugar water and flies receiving no sugar-water supplement.

The results of this study (fig. 3) indicated how essential sugar water is in the diet of confined flies. Less than 40% mortality occurred among flies supplied with sugar water during the 96-hour test, whereas 100% mortality occurred among flies not provided with a sugar-water supplement to the diet.

The efficiency of this method of rearing leaf miners was emphasized by the multiplication of less than 100 flies to more than 10,000 over a period of three generations.

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Dorilas (Eudorylas) subopacus (Loew), a Parasite of the Leafhopper, *Scaphytopius acutus* (Say)[1]

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The leafhopper, *Scaphytopius acutus* (Say), is one of the principal vectors of the western X-disease virus of peach, cherry, and chokecherry in the state of Washington. For the past 10 years from June to September, both nymphs and adults were often collected from alfalfa in the field and brought into the greenhouse to start colonies in rearing cages. Since 1953, a dipterous parasite often appeared in the rearing cages. This insect was identified by Dr. D. Elmo Hardy of the University of Hawaii as *Dorilas* (*Eudorylas*) *subopacus* (Loew), a species of big-eyed flies. Although Dorilaidae are known to have a wide distribution and commonly parasitize leafhoppers, this is the first host record for *D. subopacus* according to Dr. Hardy (unpublished data). The female *Dorilas* has a long, slender ovipositor used for inserting the eggs inside the abdomen of the host. The larva grows to maturity inside the leafhopper and, when full grown, fills the abdomen and often part of the thorax. The mature larva breaks out of the abdomen, killing the host, and drops to the ground for pupation (Hardy 1943).

Table 1.—Number of adult *Dorilas subopacus* reared from 25 adults and 25 nymphs of *Scaphytopius acutus* collected during June. Wenatchee and Monitor, Washington.

CAGE NO.	NUMBER OF PARASITES			
	1953	1954	1955	1956
1	4	3	8	2
2	2	7	2	6
3	4	2	1	4
4	1	6	1	4
5	6	7	6	2
6	5	1	4	1
7	2	5	4	3
8	0	5	3	2
9	3	3	2	1
10	6	3	5	1
Total	34	42	36	26

In checking the source of parasitized *S. acutus* it was found that they were collected from only one definite area between Monitor and Wenatchee, Washington. During the second week of June of each year from 1953 to 1956, leafhoppers were collected from various locations in the Wenatchee Valley and placed in cages where they were held, long enough for the parasites to reach the adult stage of their life cycle. This was done in an attempt to determine if the parasites were spreading rapidly and to determine if parasitism was on the increase in the known infested area.

Dorilas parasites were recovered from only the known infested area. Twenty-five *S. acutus* adults and 25 nymphs, collected from this area, were placed on celery plants in each of 10 cages during June of each year. Adult parasites appearing in the cages each day were removed with an aspirator and their numbers were recorded. The greatest number of adult parasites emerged after the leafhoppers had been caged for approximately 1 month. After the cages had been observed for 2 months, the tests were discontinued and the total number of parasites taken from each cage of 50 leafhoppers was recorded (table 1).

In 4 years no evidence was found that the *Dorilas* parasite of *S. acutus* was spreading to new areas or that the known infested area was becoming more heavily populated with the parasite. The population of *S. acutus* in the infested area also remained approximately the same during that period.

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[1] Scientific Paper No. 1642, Washington Agricultural Experiment Stations, Project No. 848. Accepted for publication August 12, 1957.

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Clinical Test of the Efficacy of Dow ET-57[1] for Grub Control in Cattle[2]

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Losses to the livestock industry caused by the larvae of the cattle grubs *Hypoderma lineatum* (De Vill.) and *Hypoderma bovis* (L.), are estimated to be in the neighborhood of one hundred million dollars annually and constitute one of the major losses to the cattle industry. Records show that grubs may cause losses as high as $35 per head due primarily to hide and carcass damage.

The answer to this costly problem is elimination of the adult stage of these flies. Heretofore grub control has been aimed at larval stage control because of the complexity of destroying the fly itself. Control programs aimed at treating all animals in an area have not been entirely successful. This has been due to the failure of treating all cattle within the area, plus the problem of infestation from untreated cattle at the borders of the area.

Rotenone has been the insecticide of choice for grub control. However, the necessity for careful timing of applications and its incomplete effectiveness creates the desire for a more effective and efficient insecticide. A test utilizing a systemic insecticide composed of an organic phosphate called Dow ET-57, manufactured by the Dow Chemical Company is reported in this paper.

To determine effectiveness of this specific systemic insecticide 154 head of Montana and Colorado yearling steers were made available December 20, 1956 by the Rath Packing Company, Waterloo, Iowa. On December 28, 1956, 77 of the animals were treated with a dose of 110 milligrams per kilogram of animal weight. (Approximately 5 gm. per 100 pounds body weight.) Gelatin capsules containing the insecticide were administered through the use of a balling gun. The animals were then separated into different lots and were provided with identical rations, management and housing.

The treated animals showed evidence of depression and inappetence within 6 hours and did not resume full feed intake for 24 to 36 hours after treatment. Three of the treated animals, showing extreme weakness, depression and apparent weight loss, were isolated from the group for 3 days. They were not treated for the symptoms and when returned to the group, resumed normal feed consumption.

Grub counts by palpation on the live animals were made 49 and 84 days after treatment. Although a severe grub infestation was not evident, no grubs were found on the treated animals as compared with an average of 2.2 grubs per animal on the controls. When the animals were slaughtered 106 days after treatment the carcasses of the 77 treated animals carried 44 grubs as compared with 194 in the carcasses of the untreated animals. Hide examination showed five grubs in the treated group and 370 in the untreated group. Accumulative anti and post mortem grub counts totaled 49 on the treated and 911 on the controls, giving 94.7% grub control (table 1).

The treated group gained an average of 264 pounds per head during a 106-day feeding period compared with a 245-pound average gain in the controls (table 2). Average daily gains were 2.48 pounds and 2.33 pounds for the treated and control groups, respectively. Feed per 100 pounds of gain averaged 979 pounds per animal for the treated group compared with 1,033 pounds per animal for the controls. The cost per 100 pounds of gain amounted to $27.15 in the treated group and $28.62 for the controls. The treated animals averaged 19 pounds more gain per animal than the controls.

The results obtained in weight gain and feed conversion need to be compared with other trials to determine their significance. There was no appreciable difference in the grades of cattle in the treated and untreated groups.

Table 1.—Effect of Dow ET-57 on number of cattle grubs before and after slaughter.

	Number of Grubs Present (1957)				
	Palpation		At Slaughter April 17		
	Feb. 15	March 21	Carcass	Hide	Accumulative Total
Treated (Dec. 28, 1956)	0	0	44	5	49
Untreated	1.9	2.6	194	370	911

Table 2.—Effect of Dow ET-57 on weight gain, cost of gain, and carcass quality of cattle.

Category	No. of Head	Average Weight on 12/28/56	Average Weight on 4/27/57	Gain Per Head (Lb.)	Feed/100 Gain (Lb.)	Cost of Gain per 100 Pounds	Grade at Slaughter
Treated (Dec. 28, 1956)	77	883	1,147	264	979	$27.15	68.57% H.C.[a] 31.43% L.C.
Untreated	77	903	1,148	245	1,033	$28.67	71.71% H.C. 27.27% L.C. 1.02% common

[a] H. C., high choice; L. C., low choice.

SUMMARY.—In 77 head of cattle moderately infested with cattle grubs, Dow ET-57 administered by capsule 110 days prior to slaughter controlled 94.7% of the grubs. Better weight gains and feed conversion were observed in the treated group than in the controls.

[1] Purified *O,O*-dimethyl, *O*-(2,4,5-trichlorophenyl) phosphorothioate.

[2] Accepted for publication August 5, 1957, as Journal Paper No. J-3230 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project No. 1309.

The Effectiveness of Several Insecticides against the Vegetable Weevil[1]

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The vegetable weevil, *Listroderes costirostris obliquus* (Klug), is a pest of fall and winter vegetables in Georgia. It is especially serious and builds up to heavy infestations on turnips. The larvae feed on both the foliage and roots of this crop. As an example of the abundance of this insect, an average of 56 larvae per foot of row was found on turnips in January, 1957.

Beckham (1953) published a rather comprehensive report on the vegetable weevil covering its life history and control. At that

[1] Paper No. 317, Journal Series, Georgia Experiment Station, Experiment. Accepted for publication August 12, 1957.

time, a number of insecticides were found to be effective against this pest; however, some of these materials cannot be used on vegetable crops because of excessive residues. Since the above report, the insecticide malathion has been developed and has found wide acceptance because of its effectiveness on a wide range of insects and because residue tolerances have been established which permit its usage on many vegetable crops. The main purpose of the present investigation was to determine the effectiveness of malathion against the vegetable weevil. Since there has been much concern about insect resistance to insecticides and about the need for establishing reference points on toxicity for different insect species, the LD_{50} for a number of insecticides against the adult stage of the vegetable weevil was determined.

A heavy infestation of the larval stage of the vegetable weevil was observed during early January, 1957, in a planting of turnips providing an opportunity to test malathion along with parathion and methoxychlor. These materials, along with lindane, were also tested in the laboratory to determine their toxicity to the adults when applied topically. Lindane had previously been shown (Beckham 1953) to give excellent control of this weevil in the field.

PROCEDURE.—*Field test.*—The experiment was laid out in a planting of Seven Top turnips. One-row plots 25 feet long spaced 42 inches apart were used. The treatments were replicated four times and were arranged in a randomized block design with a guard or untreated row between each plot.

The insecticides were applied to the foliage as dust formulations with small hand dusters on January 12. One per cent parathion and 5% methoxychlor were applied at the rate of 15 pounds per acre and 4% malathion at 30 pounds per acre.

The temperature at the time of application of the insecticides was 36° F. Rain occurred on January 14 and 15, and a minimum temperature of 17° F. was recorded on January 18.

In order to evaluate the insecticides, the larvae were collected from three sample areas, each 18×18 inches, per plot. The turnip foliage was removed at the soil level, discarded, and all live and dead larvae on the soil surface were collected on January 18. At least three larval instars were present. The larvae were examined in the laboratory and scored as live, moribund, or dead. Moribund larvae were retained and observed again on January 22 and scored as live or dead. These figures were added to the original counts for the two categories. The total number of larvae observed in the counts ranged from 590 to 1,303 per treatment. The percentages of live larvae were converted to angles for statistical analysis.

Since the mature larvae leave the turnip foliage and enter the soil to pupate and complete their development, an examination was made to determine the number of larvae and pupae in the soil to further evaluate the treatments. Soil samples, consisting of 10 subsamples, were taken on February 4 with a golf hole-cutter and examined for presence of larvae or pupae. The stages observed were approximately two-thirds mature larvae and one-third pupae.

Topical application.—Lindane, malathion, methoxychlor, and parathion were tested against adult vegetable weevils in the laboratory to determine their relative toxicity. Technical malathion was furnished by the American Cyanamid Company. The other materials were Entomological Society of America Reference Standards and were acquired from the Nutritional Biochemicals Corporation. The technical materials were dissolved in acetone and applied to the dorsum of field-collected vegetable weevils. A microliter droplet was measured and applied to each weevil with a micrometer-driven syringe similar to the device proposed by Trevan (1922).

A series of six dosage levels was used for each of the insecticides. Ten weevils were used in each treatment and the treatments were replicated four times. Treated weevils were held in ½-pint ice cream cartons with screen wire tops and were provided with turnip or chickweed foliage for feed. Mortality counts were made 24 hours after treatment. The percentages of mortality were adjusted by Abbott's (1925) formula and plotted on log-probit paper, from which the LD_{50}'s were read.

Table 1.—Control of the vegetable weevil with insecticide dusts in the field. Experiment, Georgia, 1957.

TREATMENT	LIVE LARVAE 10 DAYS AFTER APPLICATION		MATURE LARVAE AND PUPAE IN SOIL
	Per Cent	Angle	
Malathion	30.2	33.24	35.8
Parathion	37.1	37.50	39.5
Methoxychlor	82.2	65.19	53.5
Check	89.5	71.15	66.3
L.S.D. at 5% level		5.95	15.00
at 1% level		8.55	21.56

RESULTS.—*Field test.*—Data presented in table 1 show that malathion and parathion were highly significant in controlling the vegetable weevil larvae and while methoxychlor was barely significant at the 5% level, the control was not adequate. According to the results of the soil examinations, the dusts which gave the best control of the larvae on the foliage also showed significant reductions of the stages occurring in the soil.

Topical application.—Table 2 shows the median-lethal dosages of the insecticides and their fiducial limits at the 5% level. Lindane, malathion, and parathion were highly toxic to the vegetable weevil. Methoxychlor, however, was relatively nontoxic at dosage levels up to 400 micrograms per weevil.

Table 2.—LD_{50}'s of some organic insecticides to vegetable weevil adults. Experiment, Georgia, 1957.

INSECTICIDE	L.D. 50's IN MICROGRAMS PER WEEVIL	FIDUCIAL LIMITS AT 5% LEVEL
Parathion	0.3	0.2– 0.4
Lindane	3.8	3.0– 4.8
Malathion	8.0	5.0–12.8
Methoxychlor	>400	

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Hibernation Sites of the Boll Weevil in Relation to a Small, Georgia Piedmont Cotton Field[1]

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The economic losses caused by the boll weevil to cotton in the South are well known. The adult weevils hibernate in many places which afford protection from the elements, and emerge in the spring and early summer to infest cotton plants. It is generally considered that weevils hide in and around old cotton fields, especially in adjacent wooded areas.

It has been stated by many cotton growers in Georgia that more weevils are usually present in the northern than in the southern part of the State. Fall and spring trash examinations have shown this to be the case 5 out of 6 years. One reason ad-

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vanced for the difference in numbers of weevils in the two areas has been that cotton fields are generally smaller in the northern part, and are usually surrounded by, or adjacent to, wooded areas. Cotton fields in Georgia are usually small in size as compared with those in some cotton-growing states. According to the 1956 records, 84% of the cotton allotments were less than 15 acres, and approximately 6% were 30 acres or more.

Bondy (1938) stated that the destruction of grass and weeds from the ditchbanks, fencerows, and turnrows, and clearing out the leaves and surface litter from the edge of woods near cotton fields are of benefit in removing hibernation quarters. He further reported that studies have shown that many hibernating boll weevils are found in the first 100 feet of woods nearest cotton fields.

Winter survival counts of boll weevils have been made in South Carolina and Louisiana for a number of years by entomologists of the United States Department of Agriculture. During recent years federal entomologists in other states as well as state experiment station entomologists have made similar counts. Studies have been in progress for the past 6 years in Georgia. Results are issued in survey reports and are useful as an indication of the number of weevils surviving to enter cotton fields as compared with previous years.

It was considered worthwhile to conduct a study of a typical small, Georgia Piedmont cotton field to determine the sites of boll weevil hibernation in and around the field and the relative numbers in each habitat. A field in Lamar County, Georgia, where cotton was grown during the 1956 season was selected for the study. An examination of the old stalks standing in the field during the winter showed considerable damage to old bolls and indicated that a high boll weevil population existed during the growing season. An examination of surface trash from adjacent woods on December 3, 1956, showed 21,780 live weevils per acre.

DESCRIPTION OF AREA.—The field planted in cotton was approximately 6.87 acres in size and was nearly square in shape. It was planted on the contour with a slope in the land to the east. The field was bordered on the south and east by woods of mostly pine and a few scattered oaks. A dirt field road ran along the west side of the field and parallel to this was a railroad track and then a main highway, beyond which was a pecan grove. To the north of the area planted in cotton was an open uncultivated field which had become overgrown with broomsedge and other miscellaneous weeds. A dense stand of pines (approximately 775 feet from the cotton) was also present to the north and beyond the open field.

PROCEDURE.—The sites or types of habitats where it was considered weevils may hide and the dates of examination were as follows: (1) surface woods trash on the south side, January 14; (2) surface woods trash on the east side, Feburary 12; (3) grass on bank of railroad fill on west side, February 8; (4) broomsedge in field on north side, January 28; (5) distant pine woods on the north side, February 8; and (6) old bolls remaining on the stalks in the field, January 29. Although it was not possible to make all the examinations at the same time, it may be noted that they were made within a 1-month period and at a time when weevils were still in hibernation. The lowest temperature recorded in the area during this period was 17° F. on January 18.

The size of the sample collected and examined in the laboratory for all types of habitats, except the old bolls, was 2 square yards. Ten random samples this size were collected in each habitat of grass on the west side and distant woods on the north side. The grass samples were collected about 50 feet from the edge of the old cotton field. The samples in the distant pine woods were collected 50 feet from the edge of the woods. Ten samples at each of the distances of 50, 150, and 250 feet from the edge of the field were collected in the woods on the south and east sides and from the broomsedge on the north. An average of 6.2 tufts of broomsedge were examined per sample. The count on the old bolls was made by collecting all bolls on the plants in 100 feet of row at 10 random locations in the field. An average of 77.5 old bolls were examined per 100 feet.

The two square-yard samples from the woods and grass areas included all surface material taken down to firm soil. In the woods areas, this consisted of pine straw, leaves, and loose soil; the latter being recovered by sweeping with a broom and shoveling into a cloth bag. The samples from grass plots consisted of dead grass and loose soil. The samples were collected and kept in individual cloth bags. These were taken to the laboratory and run through a shaking machine utilizing screens of different sizes for separating the coarse from the fine material. The trash containing the boll weevils, which would not pass through ¼-inch hardware cloth, was recovered and spread out on a heating table. Weevils emerging from the trash were collected and counted. The broomsedge tufts were pulled up, placed in bags, and carried to the laboratory and separated by hand.

RESULTS.—The average number of weevils found per acre in the different habitats and the distances from the old cotton field are shown in table 1. Examinations of the different types of habitats showed that this insect overwintered in largest numbers in surface woods trash near the old cotton field. The largest number of weevils was found in pine straw and leaves in the woods about 50 feet from the edge of the old field. Considerably fewer weevils were found at distances of 150 feet and only a few at 250 feet. No weevils were found in a dense pine woods at a distance of 825 feet, indicating a short flight range when the weevil is seeking hibernation quarters. Broomsedge was found to harbor only a few weevils 50 feet from the old cotton field and none at distances of 150 and 250 feet. Dead Bermuda grass on a railroad bank fill apparently was not sufficiently dense to afford good protection as no weevils were found in this habitat at a distance of 50 feet from the old cotton field. One interesting result of this study was the finding of live weevils in old cotton bolls remaining on the stalks in the field. Although the number of weevils found in the old bolls was low in comparison with the counts in the surface woods trash, it may be an important point in favor of stalk destruction.

Table 1.—Average number of boll weevils found hibernating in different habitats at varying distances from an old cotton field, Lamar County, Georgia, 1957.

TYPE OF HABITAT AND LOCATION	DATE EXAMINED	FEET FROM EDGE OF OLD COTTON FIELD	AVERAGE NO. WEEVILS PER ACRE
Pine woods— South side	January 14	50	7,260
		150	2,662
		250	242
East side	February 12	50	11,374
		150	1,694
		250	0
North side	February 8	825	0
Broomsedge— North side	January 28	50	242
		150	0
		250	0
Grass— West side	February 8	50	0
Old cotton bolls (In field)	January 29	0	58

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Control of *Toumeyella* Scale on Virginia Pine[1]

A. T. DROOZ[2]

In recent years a species of *Toumeyella* scale, possibly the pine tortoise scale (*T. numismaticum* (Pettit & McD.)) has caused concern among Virginia pine (*Pinus virginiana* Mill.) owners and wood-using industries in Maryland, Pennsylvania and West Virginia. Malathion had been suggested as a control for *T. numismaticum* in Wisconsin (Shenefelt & Benjamin 1955). However, these entomologists did not suggest concentrations or rates of application for ground equipment. The interpretation of previous results with malathion against *T. numismaticum* also had been complicated by the activity of coccinellid predators that decimated scale populations in unsprayed check areas.

In May 1957 an opportunity arose to attempt ground control of *Toumeyella* sp. on Virginia pine at the Shawnee State Park near Bedford, Pennsylvania. Here the host tree covers 1,500 acres of high-use land. Examinations of the area on May 14 and 15 indicated only one serious scale population covering 20 acres located ¾-mile southwest of the park office.[3] Crawlers were issuing from under the adults in great numbers at this time, and the coccinellid predator population was estimated to be one larva per 10 overwintered scales. Sooty-mold blackened the trees and several open-grown 4-foot pines were dead. The risk of immediate loss of additional trees was great, consequently plans for control were initiated.

The pine resulted from a natural seeding, and there were about 1,900 trees per acre varying from 4 to 12 feet in height, with a light scattering of taller "wolf" trees. Lanes 8 feet wide had to be cut through the stand to facilitate movement of the sprayer, and natural openings were utilized so that a heavy dosage could be deposited over a 50-foot swath.

Prespray crawler counts were made on May 20. The trees were marked for sampling in each of three parallel lines traversing the stand. Twig samples were cut and the number of settled crawlers on 1 inch of each of the 30 twigs was recorded (table 1). No prespray count was made in the control area. Observations were made in the area and indicated high crawler populations.

The insecticide consisted of 5% actual malathion emulsion in water which was applied with a Myers speed sprayer[4] at the rate of 6 gallons per acre on May 21 and 22. At this time emergence was completed and all the crawlers had settled.

A preliminary examination of the sprayed area was made May 24, at which time dead crawlers could be distinguished by their flattened appearance and the lack of the white-waxy bloom along the scale's margin. Scale mortality was heavy on trees along the sprayer's track, but scales farther in the stand appeared to be alive.

Final observations on scale mortality were conducted on July 1. No living scales were found on any trees during a general investigation throughout the stand. Dead settled crawlers could be seen on the twig samples. They had a reddish-orange, dried-wrinkled appearance. Twig samples collected in the nearby unsprayed area contained an average of 15 living female scales per inch (table 1).

A test of the effectiveness of malathion against *Toumeyella* crawlers was also conducted using a tank-type garden sprayer. One-half per cent actual malathion emulsion in water was applied in a fine spray to wet the foliage of infested Virginia pine branches on May 21. By May 24 all of the settled crawlers were dead.

Various concentrations, and rates of application of malathion to achieve control, were not tested. From the results of the tests conducted it appears that speed-sprayer applications of 5% malathion emulsion at 6 gallons per acre, or 0.5% malathion emulsion using a garden sprayer will control *Toumeyella* scale on Virginia pine.

Table 1.—Counts of settled *Toumeyella* sp. on 1 inch of twig per sample tree, Bedford, Pa. 1957.

Tree	Sample Line A	Sample Line B	Sample Line C	Live Female Scales[a]
	No. of Settled Crawlers (May 20)			
1	54	142	112	13
2	72	73	109	12
3	227	77	192	5
4	64	30	156	21
5	90	9	202	6
6	31	83	108	20
7	76	120	30	25
8	22	54	186	16
9	36	164	261	14
10	142	161	11	22
Total	814	913	1,367	154
Average	81	91	137	15

[a] In the unsprayed stand on July 1.

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[1] Accepted for publication August 12, 1957.

[2] Forest entomologist, Forest Advisory Services, Department of Forests and Waters, Harrisburg, Pennsylvania.

[3] Examination made May 14 and 15, 1957, by T. McIntyre, Entomologist, Forest Service, Northeastern Forest Experiment Station, Virginia Pine-Hardwood Research Center, Laurel, Maryland; J. Nelson and A. T. Drooz, Forester and Entomologist, respectively, with the Pennsylvania Department of Forests and Waters, Harrisburg.

[4] The tank capacity was 300 gallons with a pump pressure capable of 4,800 pounds per square inch and 16,500 cu. ft. of air per minute moved by the fan.

Pieris rapae (L.), Its Parasites and Predators in Canada and the United States[1]

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From August to November, 1956, I had the opportunity to undertake a brief survey of the parasites and predators of *Pieris rapae* (L.) in Canada (Montreal, southern Ontario, and Victoria) and in the United States of America (California). During this investigation I was unable to find certain parasites, *e.g.*, *Apanteles rubecula* Marsh., that are very common in Europe. In Germany, *A. rubecula* is one of the most important parasites of *P. rapae*. This raised the question as to whether there is any relation between the abundance of *P. rapae* in Canada and the United States, and the absence of *A. rubecula* in these countries. *P. rapae* in Europe is of lower incidence than in North America. (It was my intention to conduct rather thorough investigations in Illinois as well as in other areas of the United States; however, I was only able to initiate these studies when it became necessary for me to return to Europe.)

It was interesting to find that another parasite of *P. rapae* has much greater importance in North America than it does in Europe. I refer to *Apanteles glomeratus* (L.) which seldom parasitizes *P. rapae* in Germany but is the most common parasite of *Pieris brassicae* (L.), with infestations averaging about 60%. In the laboratory, *A. glomeratus* readily parasitizes *P. rapae*, as well as *P. brassicae*, so we do not believe that there are two different races of this parasite. Furthermore, we do not

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believe that this difference can be the result of climatic differences. Accordingly, this problem is worthy of further detailed study.

In the United States I searched in vain, in *P. rapae*, for microsporidian parasites which are very common in Europe in this butterfly as well as in other Pieridae. Paillot, for example, from 1918 to 1933 discovered in France four species: *Perezia mesnili*, *Perezia legeri*, *Perezia pieridis* and *Thelohania mesnili*. In recent years we have also found many species in Germany; for example: *Nosema polyvora* Blunck, *Plistophora* cf. *schubergi* Zwölfer, *Perezia mesnili* (Paill.) Tan., *P. legeri* Paill., *P. pieris* Paill., *Nosema* cf. *legeri* Paill. and *Thelohania mesnili* Paill. Furthermore, we also recorded the presence of *N. polyvora* in Austria (Steiermaerk) and Switzerland. In many places the percentage of caterpillars infected with this microsporidian is rather high. For this reason, we were astonished in not finding any Microsporidia in *P. rapae* in northern and central California: *i.e.*, Garberville, Healdsburg, Ukiah, Sebastopol, Santa Rosa, Los Gatos near San Jose, Half Moon Bay, Murphy, Calaveras Big Trees in the Sierra Nevada, Tracy, Santa Clara Valley, and Albany near Berkeley. No survey was made in southern California. Caterpillars sent to us by Dr. J. D. Briggs from Urbana, Illinois, and samples collected by us in Victoria, British Columbia, and in Montreal, Quebec, and near Fort Henry in Ontario, were also uninfected. Only at Guelph, in southern Ontario, did we find one out of 60 caterpillars (fourth and fifth instars) of *P. rapae* infected with what is probably *Nosema polyvora*. Unfortunately, this larva had been preserved in alcohol so that it was not possible to initiate further infections with this material. Accordingly, our determination may be somewhat uncertain. In any event, we can conclude that Microsporidia are probably much less common in *P. rapae* in North America than in Europe, and that only one species (*N. polyvora*) of Microsporidia is involved. Taking into consideration the limitation in the number of our samples, it would appear that this species might have been introduced into Canada in recent years, thus explaining its apparently very limited local occurrence.

In the autumn of 1956 we observed in California a remarkable disproportion between the butterflies and larvae of *P. rapae*. In the central part of this state the butterflies were flying during early November in numbers as numerous as in September. On the other hand, caterpillars had already become scarce during the last month of the summer. This was true even in the areas where there had been no insecticide treatments. Eggs could be found in considerable numbers up until the end of October. Caterpillars hatched and began to feed but they diminished in numbers and became rare before reaching the second or third instar. At first, from observations in a garden in Los Gatos, it was presumed that the caterpillars were unable to feed on the cabbage leaves because the latter had hardened during the dry summer. However, this assumption was discarded when I observed that in the laboratory the caterpillars could be successfully reared by feeding them these hard leaves. Moreover, they refused to feed on the tender leaves of *Raphanus raphanistrum* L., *Sinapis arvensis* L., and *Brassica campestris* L. We suspect, therefore, that predators probably killed the larvae before they could mature. Indeed, a *Formica* sp. was observed in a small sandpit feeding on young and old caterpillars of *P. rapae*. In the laboratory, however, workers of this ant refused to feed on caterpillars and eggs of *P. rapae*. It is possible that Carabidae and *Forficula* sp. are also effective predators. However, since they are active at night I was not able to prove this supposition.

In Montreal and elsewhere pupae of *P. rapae* were effectively parasitized by *Pteromalus puparum* L. Here and in California I often found tachinids which parasitized pupae, but only one species was bred and not yet determined. According to Chittenden (1916) *Exorista bulgaris* Fall. [Tach.], *Frontina archippivora* Wills. [Tach.] and *Bottcheria latisterna* Parker [Sarcoph.] are known as parasites of *P. rapae* in the United States.

We did not observe any of the numerous Ichneumonidae and Chalcididae living as hyperparasites in *A. glomeratus*. In Germany these are frequent and diminish the useful effect of *A. glomeratus*. According to the literature, *Tetrastichus rapo* Walk. has been found in North America.

Insecticidal dusting powders (DDT and others) used to control *P. rapae* have an unfavorable effect on *A. glomeratus*. In California in areas where no insecticides were used, 20 to 50%, and sometimes as high as 60%, of *P. rapae* were found parasitized by *A. glomeratus*. On the other hand, in fields to which insecticides had been applied a few weeks previously, only a small per cent (not more than 6%) of the caterpillars were parasitized. It would appear, therefore, that *A. glomeratus* is killed by the insecticides to a high degree and that this braconid returns slowly to the fields while the butterflies return more rapidly.

I wish to express my thanks to Drs. J. D. Briggs (Urbana), B. D. Burks (Washington), T. R. Gardner (Beltsville), E. A. Steinhaus (Berkeley), and Y. Tanada (Berkeley) for the friendly manner in which they furthered my work.

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An Analysis of *Stomoxys calcitrans* (L.) for Vitamin A[1]

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Vitamin A is an essential nutrient requirement for most vertebrate forms but its role in the invertebrates, particularly the Insecta, has not been fully established. From the researches of most workers vitamin A does not appear to be present in most insects nor is it a dietary necessity (Levinson 1955, Roeder 1953). It is known, however, that this compound is present in many arthropods (Fisher *et al.* 1952) and in some it is believed to be a factor in their visual system (Kampa 1955).

In a study of the photoreceptors of a laboratory colony of *Stomoxys calcitrans* (L.) a number of flies were analyzed for the presence of vitamin A.[2] Two adult *Stomoxys* populations, of 2,500 and 3,500 flies, respectively, were ground in a mortar and pestle. The first population was provided with 0.5M dextrose for 24 to 48 hours after emergence and then macerated; the second was provided with beef blood for approximately 96 hours after emergence and then starved 24 hours before maceration. The 24 hours' starvation of the latter population and the diet of 0.5M dextrose in the former were imposed so that no blood would be in the gut.

Both populations and a control known to contain vitamin A were then extracted for the vitamin according to the following procedure:

The samples were refluxed in 500 milliliters of hexane for 30 minutes after which the hexane was filtered off and 10 grams of anhydrous sodium sulfate added to the filtrate. The hexane was poured off and evaporated to dryness. To the residue were added 30 milliliters of absolute alcohol and 3 milliliters of 50% potassium hydroxide, then refluxed 20 minutes, cooled and 30 milliliters of water added. This was extracted once with 90 milliliters of ether and the aqueous portion discarded. The ether was then rinsed with water until the aqueous portion was no longer alkaline to litmus. Three grams of anhydrous sodium sulfate were then added to the ether phase, the material shaken and the ether decanted. This was then evaporated to dryness and 2 milli-

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[2] The author wishes to thank Dr. A. J. Forgash of Rutgers University for his suggestions regarding the vitamin A analysis.

liters of chloroform added. To 1 milliliter of the chloroform solution 10 milliliters of antimony trichloride were added—if vitamin A is present the solution becomes blue in color.

The results obtained for both populations were negative. The control was positive. The lower limit of the analysis used was 1×10^{-6} milligrams per fly. This indicates that, for all practical purposes, vitamin A is not present when whole flies are extracted, and supports the general thesis that vitamin A is not necessary to or manufactured in most insects. It also indicates that the vitamin is lacking before as well as after a blood meal, since the population fed only 0.5M dextrose and the one fed beef blood and starved were both negative.

It should be emphasized, however, that this research does not necessarily mean that vitamin A is absent in *Stomoxys calcitrans*. Fisher *et al.* (1952) found that the eyes of the Crustacean, *Euphausia pacifica*, when analyzed separately from the body, had a high concentration of vitamin A, but no vitamin A was present when the whole specimen was analyzed. These authors postulate that the body of this form may contain some factor that causes the destruction of vitamin A. This could well be the case in the Insecta in which an analysis of the whole insect did not reveal vitamin A, but an analysis of merely the head might reveal it.

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A Sugarcane Leafhopper Damages Wheat in Northern India[1]

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The sugarcane leafhopper, *Pyrilla perpusilla* (Wlk.),[3] is a serious pest of sugarcane in India. The adults and nymphs both suck up the juice of the plant and the yield of sugar from the cane is very much reduced (Gupta 1955). With the advent of the better varieties of sugarcane and improved agricultural practices this insect which was so far considered as a cyclic pest is now becoming a regular menace to the sugar industry. The practice of applying heavy doses of nitrogenous fertilizers to obtain bigger cane yields is also responsible for its change in status. Srivastava (1957) showed that there was a significant correlation between the rate of nitrogen and the population of these leafhoppers in sugarcane. The population increases with the increasing doses of nitrogen applied to the crop.

The uncontrolled population of this pest becomes very high at the time of the harvest of the sugarcane crop (December–January). This has recently resulted in the migration of these leafhoppers from the harvested sugarcane crop to the adjoining wheat fields. During this period the wheat crop comes into the milk stage of grain formation and serves as a very favorable food for these insects. Large numbers of these leafhoppers have been observed on the earheads of the wheat crop. Due to the sucking of the milky juice by a large number of individuals the grains become shriveled. The earheads of badly infested wheat crop show numerous grains sucked empty which turn brown after drying. Ears which fail to bear grains turn white. The author had noticed for the first time the damage done by this pest to wheat in the Jarwal Road Sugar Factory area in 1951. When this fact was brought to the attention of all those concerned with crop pest control operations and the State Entomologist, it was difficult for them at that time to believe that this insect could also cause injury to the wheat. This was the first record of this pest damaging wheat. Since then attacks of the sugarcane leafhopper on the wheat crop have gradually increased and are well known. The ravages of this pest are now becoming widespread and several cases have been reported where the wheat had suffered great losses ranging from 20% to total failure of the harvest.

Wherever the sugarcane leafhoppers are present in appreciable numbers on the wheat crop during the milk stage of the grain it has become absolutely essential to control the infestation in order to save the crop. BHC has been tried against this pest on the wheat crop and it was found that 5% dust applied at the rate of 15 pounds per acre effectively controlled the pest.

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[1] Accepted for publication September 9, 1957.

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[3] Homoptera: Fulgoroidea: Lophopidae.

Effect of Endrin Sprays for Mouse Control on Insects Found on Orchard Cover Crops[1]

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Orchardists are showing an increasing interest in the use of endrin as a cover crop spray to control orchard mice (*Microtus*). In Virginia, Horsfall (1955, 1956) has demonstrated that endrin sprays are more effective than any other mouse control measure yet tried in orchards. Since 1953, experiments have been conducted in central Washington with endrin[3] for control of mice in orchards. Most sprays were applied from mid-September to mid-November. Observations were made in the experimental plots on the effects of sprays on the insect fauna of alfalfa cover crops, especially where lygus bugs, stink bugs, etc. have caused stigmonose injury or catfacing of fruit.

Several experimental plots were sprayed in a peach orchard where such insects were abundant in the alfalfa cover. Both handgun-type and airblast-type sprayers were used. Where handgun equipment was used the entire cover crop was sprayed; however, where the airblast machine was used, an area between the wheel tracks was left unsprayed. From 1.2 pounds to 2.4 pounds actual endrin was used per acre as emulsion in 300 gallons of water. Counts were made by net sweepings in the alfalfa cover before and after spraying. Sweeps were made in a pattern across rows so that sampling would include the unsprayed strips where airblast equipment was used. All lygus bugs, stink bugs, leafhoppers and aphids were counted. The species of lygus bugs involved were *Lygus hesperus* Knght. and *L. elisus* Van D. Only one species of stink bug, *Euschistus conspersus* Uhler, was found. Leafhoppers were the geminate leafhopper (*Colladonus geminatus* (Van D.)), the mountain leafhopper (*Colladonus montanus* (Van D.)), *Scaphytopius acutus* (Say), *Dikraneura carneola* (Stal.) and *D. absenta* DeL. & Cl. The pea aphid,

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[2] Now Entomologist, U. S. Dept. of Health, Education, and Welfare, Public Health Service, Communicable Disease Center Activities, Wenatchee, Washington.

[3] Endrin emulsible concentrate supplied by Shell Chemical Corporation and Velsicol Chemical Corporation.

Table 1.—Number of certain sucking insects on alfalfa orchard cover crop before and after treatment with endrin sprays for meadow mouse control. Wenatchee, Washington. September 1956.

Pounds Per Acre	Amount of Cover Crop Sprayed	Number of Insects per 100 Sweeps[a]							
		Before Treatment				48 Hours after Treatment			
		Lygus Bugs	Stink Bugs	Leaf-hoppers	Aphids	Lygus Bugs	Stink Bugs	Leaf-hoppers	Aphids
1.2	Entire[b]	957	40	167	1756	1	0	2	0
1.8	Do.	920	33	170	1812	0	0	0	0
2.4	Do.	887	13	89	1430	0	0	1	0
1.2	Tree rows[c] only	726	17	85	1067	2	0	16	440
1.8	Do.	560	10	66	996	6	1	14	468
2.4	Do.	652	5	59	1026	12	0	3	302

[a] One full stroke with a net 15 inches in diameter with handle 3.5 feet long was counted as 1 sweep.
[b] Handgun-type application.
[c] Applied by airblast machine, leaving area between vehicle wheels unsprayed.

Macrosiphum pisi (Harris), was the principal aphid species found. The data presented in table 1 indicate good kill of these insects in plots where the entire cover crop was sprayed by handgun, and a great reduction of insects where the cover in the tree rows only was sprayed by airblast machine.

No study was made on the effect of the sprays on insect life hibernating in the orchard ground cover. In Virginia Hough (1957) found that endrin mouse-control sprays had very little, if any, effect on certain overwintering Lepidoptera or their Hymenopterous parasites.

Although endrin sprays for orchard mouse control in Washington are applied during the fall season after harvest, these control measures, if practiced each year, should prevent a heavy build-up of certain sucking insects, resulting in less damage to fruit the following year.

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Ornithonyssus bursa (Berlese) Attacking Man in Puerto Rico[1]

Irving Fox,[2] *School of Tropical Medicine, San Juan, P. R.*

The attack of man by bird mites is of epidemiological significance because such mites are implicated in the transmission of arthropod-borne encephalitides. Since *Ornithonyssus bursa* is recorded as giving positive results in attempts to isolate the virus of western equine encephalomyelitis from nature by Ferguson (1954), it seems worthwhile to report an outbreak of this species in Puerto Rico.

A heavy infestation of these mites occurred on the maintenance docks at an Air Force Base in Puerto Rico in late June, 1957. They were found in great abundance on locker tops, work benches, walls and even on the wings of airplanes. Workers on the docks complained of considerable annoyance because the mites crawled over clothing and skin. About 10 persons were involved in the incident of which one was definitely bitten but he did not show severe reactions, such as excessive edema or dermatitis.

Birds locally known as the "chango" (the Puerto Rican grackle, *Holoquiscalus niger brachypterus* (Cassin), according to Danforth, 1936) were very abundant on the docks and even nesting in some places. The infestation was definitely traced to the birds when in July a moribund nestling was found on the docks, which was parasitized by hundreds of mites in various stages of development. Baker, *et al.* (1956) record the species from the Bahamas, the Canal Zone, Colombia and the United States in the Western Hemisphere. Dr. Baker, to whom thanks are due for his valuable assistance in determining the species of mite, stated that he had no previous record of the species in Puerto Rico.

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[2] 1st. Lieut. MSC Ralph S. Daniels and T/Sgt. William B. Lubinsky, U. S. Air Force Hospital, Ramey Air Force Base, Puerto Rico, assisted with the observations reported in this paper.

EDITORIAL

Common Names for Pesticides

Among the problems that attend the development and introduction of a new pesticidal chemical is the choice of a common name by which it may become known among scientific workers and to the public. Common names have arisen in different ways. Many were adopted long before the chemical constitution of the compounds was known. Frequently they carry some connotation of the source of the product or its properties. Such names are nicotine, carbolic acid, indigo, and urea. Others, such as acetin and sulfapyridine, are simplifications of scientific names and are usually related in some way to the technical names. Combinations of letters and numbers taken from component groupings of the compounds have been used, but they are not desirable for several reasons. Both letters and numbers are objectionable to libraries from the standpoint of indexing. DDT has on occasion been confused with the soil fumigant DD. For many years monocalcium phosphate, which is used both as a drug and in foods, has been designated MCP. Several years ago the same designation was adopted for a weed killer. In the early stages of its development the insecticide now known as chlordane was designated as 1068. In at least one instance during World War II, it was confused with the very toxic rodenticide 1080.

For several years the Committee on Insecticide Terminology of the Entomological Society of America has compiled a list of common names of insecticides for use in the Journal. This list is confined to the principal organic compounds that have been developed as insecticides in recent years, the chemical names of which are too long for convenient use. The most recent revision was published in Volume 50, No. 2, pp. 226–8 (1957) of the Journal.

It is not always necessary to adopt a common name for a new pesticidal chemical. When such a product has a short convenient technical name, it seems preferable to use it, especially since chemical terminology is rather universally understood. When a new product has a long and complicated technical name, however, a common name is useful to both the scientific worker and the lay public. Such a name should be chosen with care. It should be easy to pronounce, but should not be conducive to formation of abbreviations or nicknames and it should conform to the principles of nomenclature in regard to ending. The latter provision is important because the ending of a chemical name is often indicative of the class of compounds to which it belongs.

The problem of having a short name of a type called variously "generic," "nonproprietary," and "coined common" name is essentially not a new one. It has received attention in other fields, especially for pharmaceuticals, as early as 1911.

Until World War II there was little need for an organized effort to coin common names for pesticidal chemicals because relatively few new ones reached the commercial stage of use. The technological revolution in the pesticidal chemical industry, fostered by World War II, brought forth a large number of new products. Most of these were synthetic organic chemicals with complicated structures and unwieldy systematic names. Attention was soon focused on the advantages of having short names that could be easily remembered by technicians and users. To the late S. A. Rohwer must go the credit for pioneering an organized attempt in this direction. In April 1947 Dr. Rohwer as chairman of the newly formed Interdepartmental Committee on Pest Control, a group representing the various departments of the Federal Government, approached several scientific groups having an interest in pest control chemicals and solicited their cooperation. Among those who responded promptly and cooperated fully were the American Medical Association, the American Phytopathological Society, the American Chemical Society, the former American Association of Economic Entomologists, and industry. The project was well received, and between 1947 and 1951 names for eight insecticidal chemicals, five fungicides and one rodenticide were coined. The international aspects of the problem were recognized by Dr. Rohwer, who sought the cooperation of Canadian and British workers.

In the fall of 1953 the Interdepartmental Committee on Pest Control discontinued the approval of coined names for pesticidal chemicals because it was organized primarily as a coordinating group and not as an operational one. It suggested that the American Standards Association be asked to initiate a national standards project on the subject. Following several meetings of interested groups, a formal committee known as the American Standards Association Sectional Committee K62 on Common Names for Pest Control Chemicals was established on February 25, 1954. The scope of the Committee is to develop common names for chemicals having potential use as pesticides, including insecticides, fungicides, rodenticides, herbicides, nematocides, defoliants, and plant-growth regulators where such names appear desirable. The U. S. Department of Agriculture agreed to assume administrative sponsorship of this Committee. American Standards Association's staff cooperated fully in the organizational and procedural problems arising from the work. The Committee is comprised of the following organizations:

American Chemical Society
American Medical Association
American Phytopathological Society
American Society for Horticultural Science
American Society of Plant Physiologists
Association of American Pesticide Control Officials
Canadian Standards Association (Liaison)
Chemical Specialties Manufacturers Association, Inc.
Entomological Society of America
Interdepartmental Committee on Pest Control
National Agricultural Chemicals Association
U. S. Trade Mark Association
Weed Society of America

H. L. Haller, Assistant to Administrator, Production Research, Agricultural Research Service, United States Department of Agriculture, is chairman, and C. E. Hilton, American Standards Association, is secretary of this committee.

The Committee's "American Standard Procedure for

the Acceptance of an American Standard Common Name for a Pest Control Chemical," was approved February 28, 1956. Essentially, this procedure consists in the selection of a common name by the sponsor of the chemical, preparation by the sponsor of a statement giving the properties of the chemical and such other information as may be required, submission of the statement of the Committee, review and action by the Committee, and adoption of the common name and its release as an American Standard by ASA.

The Committee realizes that it is exceedingly difficult to coin common names that do not infringe or encroach on proprietary and nonproprietary names in various fields. It is also aware of the difficult problem of preventing trade-marking of a name in a foreign country which has hitherto accepted this name. However, it believes that agreement on suitable terminology can come only through cooperation of the various groups concerned. A greater exchange of information, standards, proposed terms, and other pertinent data by responsible agencies, both here and abroad, should bring about the desired results.

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BOOK REVIEWS

INDEX XV TO THE LITERATURE OF AMERICAN ECONOMIC ENTOMOLOGY, 1955. Compiled by INA L. HAWES, Library, U. S. Department of Agriculture, 8 vo., cloth, 336 pp., Washington, D. C. Entomological Society of America, Special Publication 15. 1957. $3.00.

All who perform research in entomological bibliography are glad to welcome each year the latest issue of this well known reference work. This time, however, our satisfaction is mingled with sorrow. Miss Ina L. Hawes (1896–1957), Librarian U. S. Department of Agriculture, 1917 to 1957, and compiler of volumes VII to XV, covering the period 1940 to 1955 inclusive, was not able to finish preparation of printer's copy of this issue. Her last illness and death occurred while final typing was in progress. Mrs. Helene G. Cushman, of the Library Staff, supervised the typing of the last half of the copy and reviewed the entire manuscript. It will be remembered that this exceedingly useful reference work reaches back for 96 years and covers American economic literature from 1860 down to the present time. It was the urgency of the need for a work of this kind that prompted the beginning of such a compilation by B. Pickman Mann and Samuel Henshaw, and later continued by Nathan Banks. At the beginning the work consisted of eight volumes and covered the years 1860 to 1905 and was published under authority of the U. S. Congress. The volumes subsequent to that period were prepared by Nathan Banks, Mabel Colcord and Ina L. Hawes, assisted at various times by other interested workers. Beginning with Index VII, it became necessary to enlarge the geographical scope of the series to comprise continental North America, including Canada, Alaska, Mexico, Panama Canal Zone, Cuba, Puerto Rico, Hawaii, and certain other Pacific Islands, particularly those that played a part in World War II. This Index covers in minute detail a field of research not fully or adequately covered elsewhere, therefore it would be a matter of difficulty to attempt to evaluate its outstanding usefulness to research workers within the scope of its subject matter. This would be particularly true with regard to keeping up with the most recent periodical literature dealing with down-to-the-minute work on such subjects as the newer insecticides, the latest approved methods for their application, or, perhaps on latest results obtained from tests of new compounds and the like. Indeed, so rapid have been developments along some of these lines that it becomes of deep interest merely to note from these something of the extent to which investigations have been broadened from volume to volume in the use of new words, representing names of new compounds, new subject headings and new types of equipment, additions to the vocabulary that represent definite milestones in the advancement of our knowledge. Because of the growing multiplicity of cross references to chemicals and their duplication in the various chemical sections of the Index, these references in Vol. XIV *et seq.*, have been removed from the text and placed as a separate unit at the end of each volume, with footnotes to refer the student to this cross reference list. The Insect Identification and Parasite Introduction Section and the Pesticide Chemicals Research Section of the U. S. Agricultural Research Service have been consulted. Other Sections also have given advice in their subject fields. On the whole, this book represents an exceedingly helpful addition to a series that makes more readily available the literature on control of our insect enemies—control work that affects the welfare of millions of people everywhere.

J. S. WADE

EXPERIMENTAL DESIGNS. SECOND EDITION. William G. Cochran and Gertrude M. Cox. John Wiley, New York and Chapman and Hall, London. 611 pp. $10.25.

The application of experimental statistics to research problems in entomology has continued to increase since the first edition of "Experimental Designs" appeared in 1950. The first edition quickly found a place among the reference books of many research entomologists. Most entomology departments now require that their graduate students take one or two semesters of advanced biometry. The first edition has frequently been used as a text in advanced college courses for students in agricultural statistics. "Experimental Designs" undoubtedly had much to do in stimulating greater usage of statistical methods, opening up new avenues of approach in the matter of design, and contributing to a higher quality of workmanship among entomologists.

The second edition adequately fulfills the original desire of the authors to make available a detailed account of the most useful experimental designs, their application and analysis. Recent advances in statistical methods are included without disturbing the general framework of the commendable first edition. However, even with a substantial increase in size, it was not possible to include all of the new designs or plans in complete detail. Nevertheless, clear citations and greatly expanded references are given where additional information may be desired.

Two chapters concerning factorial experiments have been added. Fractional replication of factorial experiments is discussed in Chapter 6A. Experiments of this type have proved productive in exploratory research where an investigator can discover more quickly, but with some sacrifice in precision and a greater chance of misinterpretation, which of several factors have an important effect. Methods and designs for experiments in which factors represent quantitative variables, measured on a continuous scale, are treated in Chapter 8A. Linear or quadratic equations are employed to approximate the function within the limits of the experimental region which relates response to the

levels of the factors under consideration. Procedures are given for quick determinations of the levels of several factors required to obtain maximum response.

A table has been added for estimating the number of replications needed for comparing two treatments when the data are arranged in two classes, as dead or alive. Methods of analysis of data of this type are given for completely randomized and incomplete block designs. This addition should prove highly useful to entomologists, particularly laboratory investigators.

Another very interesting new addition concerns adjusting for residual effects which may be present when the treatments are applied in sequence to the same subject in latin squares. Other new topics include sequential experimentation, the testing of effects suggested by the data, the problem of making several tests of significance in the same experiment, Yates' automatic method of computing factorial effect totals, additional standard error formulae for split-plot experiments, the effects of errors in the weights on recovery of inter-block information, and the use of balanced incomplete block designs in taste and preference testing. New variations of incomplete block and latin square designs are presented.

Although the statistician is willing and able to assist us with our problems, he is at the same time limited to do so by a lack of knowledge of the many diverse and complex situations existing in those many fields to which statistics has much to contribute. It is expected that each scientific investigator will continue to choose the most appropriate design and analysis. In general, the use of design and analysis neither simplifies nor complicates our tasks. It simply adds a highly desirable degree of mathematical certainty to our interpretations.

Like men on a jury, it is for each of us to determine whether or not a fact has been established beyond reasonable doubt. Design and analysis can supply the same unbiased witness in every case. I believe that you, the jury, will welcome this newly revised edition to our statistical court. It is recommended for a prominent place at each of your trials.

WALTER J. MISTRIC, JR.

GENERAL AND APPLIED ENTOMOLOGY, by V. A. Little. viii+543 pp., 326 figs. Harper & Brothers, Publishers, New York, 1957. $7.00.

The trouble with entomologists who teach is that most of us were given our heads too early and let gallop off into our own specialized little bailiwicks. The result is that the profession is so infested with individualists that no teacher who has not published his own text can find one which quite suits him. We are often so determined to cram our students' heads with taxonomy, phylogeny, biology, toxicology, or whatever our specialty happens to be, that the textbook becomes merely a bit of parallel reading. This problem was unimportant as long as Comstock and Imms were the only texts in the field worth considering. Both of them were so long and so difficult to read that we were perhaps justified in assuming that our lectures would be a better guide for the beginner. In recent years, however, the situation has changed. So many new books, presenting widely different approaches, have appeared, that the teacher of entomology ought to reconsider not only his textbook but his course as well to make sure that he is getting the maximum use out of the available resources.

The present book must be evaluated with reference to the author's avowed intent of introducing the subject of entomology to the beginning student while at the same time holding his interest and furnishing him with a background of useful information. To accomplish this Dr. Little integrates general entomology with economic aspects which will allow the student to make his own immediate application. Two very large areas of entomology are thus united, and the consequent brevity of the discussions of certain aspects of taxonomy and biology cannot be too severely criticized. My general impression is that this book will serve the needs of many beginning courses extremely well. It is well written in a style which should encourage the student to read whole sections rather than browse at random.

The general arrangement and content is orthodox. Brief opening chapters on insect morphology, physiology, and metamorphosis are followed by chapters on control measures and insecticides. The bulk of the book comprises discussions of the insect orders with special emphasis on the families containing insects of economic importance. A list of the general characters placed at the beginning of each order is a useful innovation. The family keys are brief, but their limitations are clearly indicated. A chapter on collecting methods, a brief glossary of technical terms, and a selected bibliography conclude the work. An adequate index is also appended.

The many fine photographic figures are one of the admirable features. The line drawings are less conventionalized and not as "slick" as in some competing books, but many have a refreshing newness.

Dr. Little has made good use of his many years of teaching in preparing this synthesis. His book is a valuable addition to our lists and should prove particularly useful in the south and midwest.

FRANK N. YOUNG
Indiana University

NOTICE TO AUTHORS

On the inside of the front cover of the September, 1957, issue of the *Bulletin of the Entomological Society of America* (Vol. 3, No. 3) the policy of omitting the footnote designating papers for which part of the publication costs are paid (unless authors of such papers requested that it be included) was announced.

At the Annual Meeting held at Memphis, Tenn., Dec. 2–5, 1957, the Governing Board of the Society adopted a resolution requiring that papers published in the *Annals of the Entomological Society of America* and in the JOURNAL OF ECONOMIC ENTOMOLOGY heretofore carrying the footnote "Paid paper" be identified in the future by the following footnote: "Partial cost of publication of this paper was met by . . . ." (Appropriate information on agency, institution, etc., to be inserted.)

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KELTHANE
A miticide for use on fruits, vegetables, and ornamentals. Its long residual activity and low toxicity make it particularly useful.

PERTHANE
A safe effective insecticide for use on various fruit and vegetable crops. Its low range of toxicity makes PERTHANE especially useful in late season applications. PERTHANE is also effective in household sprays and mothproofers.

RHOTHANE (DDD or TDE)
This insecticide is widely used for control of horn worms, red-banded leaf roller and many other pests of fruits and vegetables.

KARATHANE
fungicide. This is the first organic fungicide to give effective control of powdery mildew. Also has activity against several mite species.

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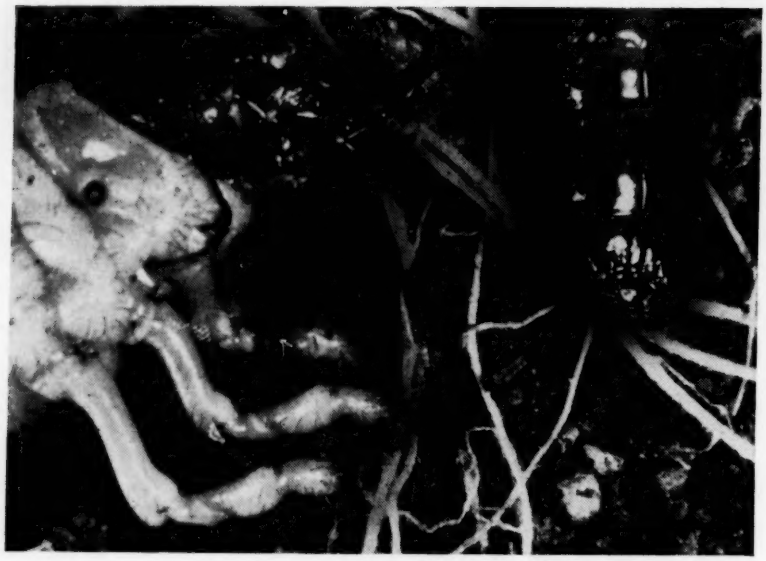
Three DITHANE products are now available. DITHANE D-14 liquid (nabam), DITHANE Z-78 (zineb) and DITHANE M-22 (maneb). DITHANE fungicides are the most versatile and widely used of any agricultural fungicide.

LETHANE
The first synthetic organic insecticide; used in many of the leading dairy and household oil-based sprays and aerosols.

TRITON
The TRITON emulsifiers and wetting agents are versatile products useful for the emulsification of pesticide formulations, as wetting agents for use in the manufacture of wettable powders, and are recommended as spreading, wetting, and sticking agents for fruit and vegetable sprays.



White grubs (left) and wireworms (right) magnified 20 times.

WHEN PESTS SEE eye-to-eye

Creatures from the science-fiction world? No, just an ordinary white grub and a wireworm magnified twenty times. But they and their equally destructive allies, such as rootworms and cutworms, ruin millions of dollars worth of cotton, corn, tobacco, wheat, cole crops and others —each season.

Constant research is necessary to find the flaws in their armor . . . to develop newer and better controls . . . to hold their crop-destruction to a minimum.

That's why Shell Chemical maintains a staff of researchers who, with the co-operation of federal, state, and local agencies, are continually searching and experimenting to develop new and more potent pesticides.

Shell's contribution to agriculture include aldrin, dieldrin, endrin, Phosdrin* insecticides; D-D® and Nemagon* soil fumigants; and allyl alcohol weed seed killer. Others that are now in the laboratory stage will be available for even more effective controls in the future. With you, we look to a better tomorrow for agriculture and industry through research in chemistry today.

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with interchangeable orifice tips

Supplied with male or female connection, in choice of over 400 interchangeable orifice tips. Give full range selection to meet any requirement. Flat spray, hollow cone, full cone and straight stream types. For all liquids including those with suspended solids. For use with boom or gun type sprayers.

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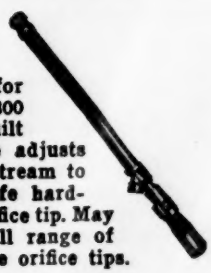
For use at any pressure up to 250 pounds. Light in weight and easily handled. Fits comfortably in hand. Positive shut-off valve with lock. For use with all TeeJet interchangeable orifice tips. Supplied with or without large capacity strainer in handle.

GunJet Spray Guns



Precision built guns for pressures from 30 to 800 pounds. Ruggedly built for hard use. Handle adjusts spray from straight stream to hollow cone. Long life hardened stainless steel orifice tip. May also be used with full range of TeeJet interchangeable orifice tips.

Spraying Systems products are sold by original equipment manufacturers everywhere as a part of the equipment they build . . . or are available directly from Spraying Systems Co. for application in experimental work. Products also include strainers and fittings required in spraying.

FOR INFORMATION . . . write for general catalog No. 30 . . . Bulletins 66 and 71, BoomJet Spray Nozzles . . . Bulletins 65, 69 and 80, GunJet Spray Guns . . . and Bulletin No. 68, Trigger TeeJet Shut-Off Valves.

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**Trademark*

After three years of extensive field tests, Delnav—a new phosphate pesticide—will be commercially available in limited quantities this year.

With indicated effectiveness in controlling a wide range of crop pests, this unusually long-lasting pesticide has already been recommended by various state authorities for use on cotton. Test results have also demonstrated the usefulness of Delnav on citrus and deciduous fruits, grapes, vegetables, and ornamentals as well as control of cattle ticks.

Developed at Hercules' Research Center and Agricultural Chemicals Laboratories, Delnav has been evaluated by Hercules, the United States Department of Agriculture, and various state agricultural research laboratories. Among the insects it controls are leafhoppers, thrips, leaf miners, and mites of various types. The effectiveness of Delnav is shown by the fact that it not only destroys adults but also the eggs of mites.

Delnav will be available in dusts or sprays in the near future. Additional information on this new product can be obtained by writing to Hercules.

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This highly adsorbent carrier has been developed especially for the processing of impregnated concentrates, both dust bases and wettable powders. Diluex A has found wide acceptance in many new insecticide developments requiring a base material capable of effectively carrying liquid and waxy organic toxicants.

East of handling, increased output, and an improved uniformity of final product commend Diluex A in grinding and blending operations.

Samples and additional data will be forwarded upon request.

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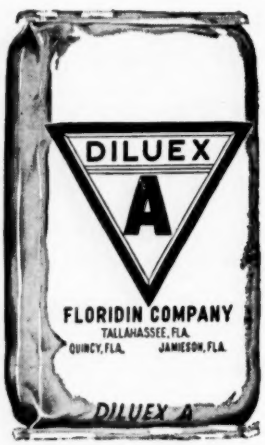
Adsorptive FLOREX granules offer a superior base for granular soil pesticides, mosquito control formulations and insecticide-fertilizer additives. Available in standard meshes 30/40, 30/60, and 16/30. Special meshes tailored for experimental formulas.



Properties of DILUEX A

Color: Light grey to cream

Composition: Aluminum magnesium silicate (Florida Fullers Earth)

Bulk: 19 to 21 lbs. per cubic foot, loose
33 to 36 lbs. per cubic foot, packed

Mesh: 90 to 95% finer than U.S. 325

Specific Gravity: 2.2 to 2.4

Surface Mean Diameter: 1.5 to 1.7 microns

pH: 6.8 to 7.1 (AOAC method)

Abrasiveness: Lowest rating

Adsorption: Adequate for impregnating liquid toxicants at recommended concentrations

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leading carriers and diluents for quality pesticide formulations at lower costs

ATTACLAY

Attaclay is a versatile carrier and diluent for the formulation of insecticides, fungicides, and other agricultural chemical dusts and powders.

Source—Attapulgite, a complex hydrated aluminum magnesium silicate—Chemical Analysis (volatile free basis)

SiO_2	67.0%
Al_2O_3	12.5%
MgO	11.0%
Fe_2O_3	4.0%
CaO	2.5%
Other	3.0%

Hardness—Degritted, non-abrasive.

Bulk Density—27-31 lbs./cu. ft. (packed condition).

Fineness—1-2 microns (surface mean diameter, by air permeation method).

pH—Neutral—no effect on acid- or alkali-sensitive materials.

Sorptive Capacity—following table illustrates Attaclay's unique sorptive properties:

Toxicant	Wt. % in Formula	Toxicant	Wt. % in Formula
Chlordane	40 and 50	Parathion	15 and 25
DDT	50 and 75	Aldrin	20 and 25
DDD (TDE)	50	Dieldrin	50
EPN	25	Endrin	25
Toxaphene	40	Dilan	25
Benzene Hexachloride	50, 75 and 85 depending on gamma content	Methoxychlor	50
		Heptachlor	25
Lindane	25	Aramite	25

Wettability, Suspensibility—Wets very rapidly, disperses readily.

Flowability—Accepts unusually large amounts of toxicants yet stays lump-free. Flows loosely during and after formulation and after long periods of storage, even under adverse conditions.

Compatibility—Physical and chemical compatibility is well established.

Dustability, Conditioning—Imparts lightness and fluffiness to dust base; adjusts volume-weight of finished dusts; conditions formulas for improved flowability and covering power.

Uses—*Grinding aid* for pesticide chemicals; *Carrier* for dust bases, wettable powders, impregnation formulas; *Diluent* for field strength dusts; *Conditioner* for finished formulas, fertilizers, and other chemicals such as ammonium sulfate.

ATTACLAY X-250

Recommended as a carrier and conditioner in those applications in which the superior sorptive capacity and fineness of Attaclay are not necessarily required. Attaclay X-250 is 96-97% finer than 200 Mesh and 100% finer than 100 Mesh, is 10-15% less sorptive than Attaclay, and has a bulk density (packed condition) of 34-36 lbs./cu. ft.

Granular ATTACLAY

Development of Granular Attaclay, based on our years of experience with granular grades, opens new opportunities for pesticide processors. For Granular Attaclay provides a most efficient means of formulating granular pesticides and applying them to the soil and to water.

Source—same as Attaclay.

Bulk Density—32-34 lbs./cu. ft.

Mesh Sizes—8/15 15/30 20/30 30/40 30/60

pH—see Attaclay.

Adsorptivity—Capacity based on laboratory kerosene sorption and commercial preparations (depending on grade): 36% to 42% Aldrin; 40% to 46% Chlordane; 33% to 39% Dilan; 40% to 46% Toxaphene; 33% to 38% Parathion. (DDT, BHC, lindane, dieldrin depend on density and concentration of solution used for impregnation.) This high sorptive capacity of Granular Attaclay permits formulation of high concentrates of toxicants without danger of caking in bags or hopper.

Formulation—Mixes well in either ribbon or rotating drum type mixer. Simple alterations to ribbon mixer practically eliminate particle breakdown. Little, if any, breakdown is encountered in drum type mixers.

Application—Shows greatest superiority over dust and emulsion formulations when applied from the air. Distribution pattern extremely uniform. Ideal for mixing granular pesticide concentrates with fertilizer to eliminate segregation. Gives excellent distribution of toxicant in soil or water. Can be used in broadcast, band or row treatment with conventional equipment.

Toxicant Release—Can be controlled by various methods. "AA" RVM Granular Attaclay disintegrates in water and tends to release toxicants faster than does the "A" LVM Granular Attaclay. Indications are that specific surfactants can control toxicant release.

Compatibility—Well established through commercial formulations.

Uses—Carrier for liquid impregnations; Soil conditioner; Anti-caking agent.

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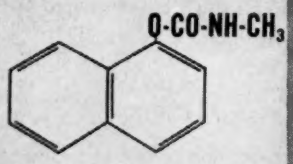
Experimental Insecticide 7744

IN YOUR PLANS FOR 1958



CRAG Sevin insecticide is a new kind of pesticide—a carbamate containing no chlorine or phosphorus. It appears to be highly effective against a wide range of insects. It's mammalian toxicity is between that of malathion and DDT. Sevin is 1-naphthyl N-methylcarbamate—the structure at the left.

In experiment station tests, Sevin insecticide has shown promise against the following:

Fruit insects: Codling moth, apple aphid, rosy apple aphid, red-banded leaf roller, plum curculio, oriental fruit moth, apple maggot, grape leaf folder, grape leafhopper, and others.

Cotton insects: Boll weevil, bollworm, pink bollworm, cotton aphid and cotton leaf perforator.

Vegetable insects: Mexican bean beetle, Colorado potato beetle, corn earworm, cucumber beetle, flea beetles, green June beetle, imported cabbageworm, potato leafhopper, southern armyworm, yellow-striped armyworm, cabbage looper, squash bug, blister beetle, and bean leaf roller.

Forest and ornamental pests: Eastern tent caterpillar, gypsy moth, bagworm, southern pine sawyer, ambrosia bark beetle, and Japanese beetle.

Pests of man and animals: Certain ticks and lice.

If you are interested in planning tests with Sevin, please write to the address below.

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Technical Development Department
CRAG Agricultural Chemicals
Union Carbide Chemicals Company
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Pyrenone* TODAY'S VERSATILE INSECTICIDE BASE

for insect control without hazard to humans and animals

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DAIRY
FOOD PROCESSING
FOOD HANDLING AND STORAGE
AEROSOLS
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Pyrenone (technical *piperonyl butoxide* and *pyrethrins*), rotenone and pyrethrum are exempt from the requirements of a tolerance under the Miller Bill. They may be used right up to the time of harvest.

Pyrenone Wheat Protectant, Pyrenone Grain Protectant and Pyrenone liquid protectants can be applied to wheat, corn (including popcorn), buckwheat, barley, rye and rough rice, in accordance with package directions, for protection during a normal period of storage with a *single* application.

UNDER THE MILLER BILL *piperonyl butoxide* has an approved tolerance of 20 p. p. m. and *pyrethrins* a tolerance of 3 p. p. m. These tolerances are in *excess* of the quantities specified for use in the package directions.

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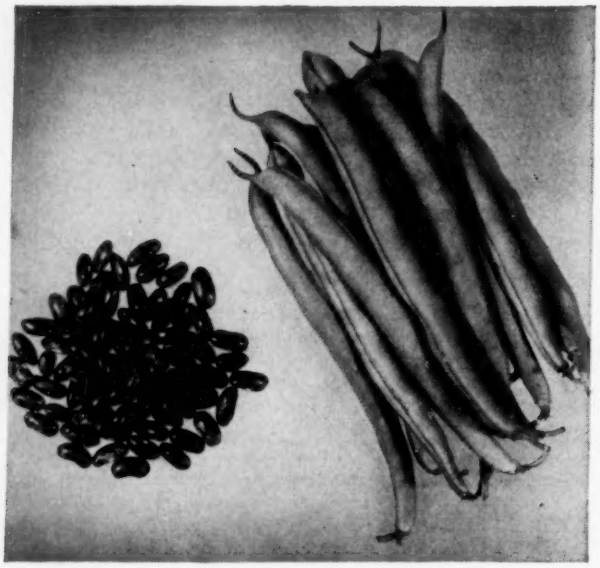

with ARAMITE

Aramite, today's safest strongest mite-killer, promises growers bigger, more profitable yields of better fruits, vegetables, row crops, cotton and ornamentals. This is possible because Aramite can effectively control a wide variety of mites at low cost per acre without harming humans, animals or crops. Further advantages of Aramite are ease of use, long residual effect, high compatibility and harmlessness to natural predators.

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POSITION WANTED: Young entomologist, M.Sci. Experienced in food products entomology and economic biology. Desire work in related field, pest control, or research. Reply Box C, Entomological Society of America, 1530 P Street, N.W., Washington 5, D.C.

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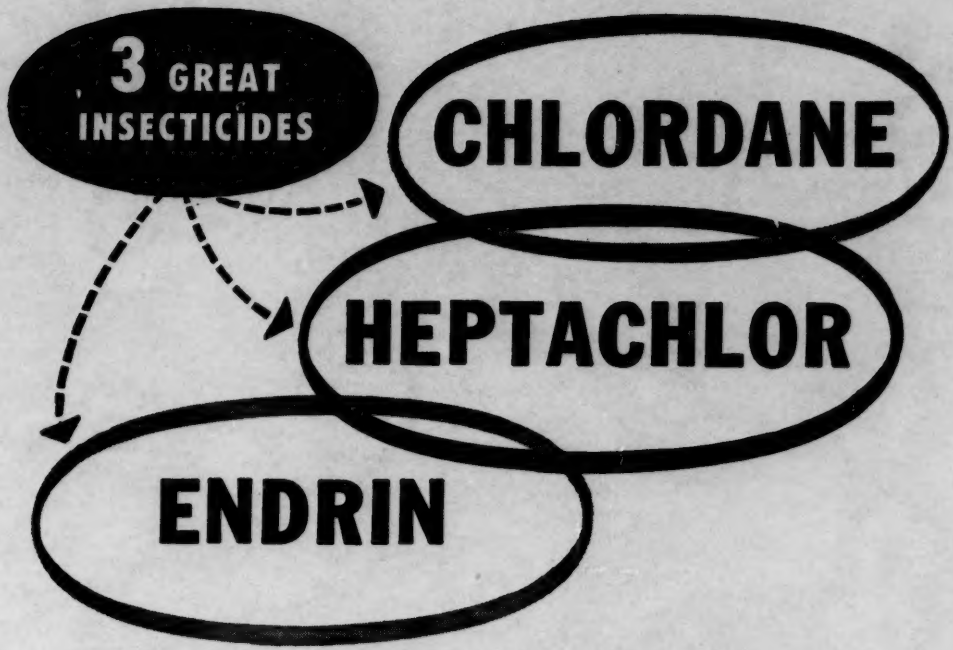
The prepublication announcement of the ANNUAL REVIEW OF ENTOMOLOGY, Volume 3 is again carried on the inside back cover of this issue of the JOURNAL OF ECONOMIC ENTOMOLOGY. All member orders are to be placed with the Society.

Index XV to the LITERATURE OF AMERICAN ECONOMIC ENTOMOLOGY, and the HISTORY OF ENTOMOLOGY IN WORLD WAR II are available at our Washington office.



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HEPTACHLOR: Alfalfa Snout Beetle, Alfalfa Weevil, Ants, Argentine Ant, Armyworms, Asiatic Garden Beetle Larvae, Black Vine Weevil, Root Maggots, Clover Root Borer, Colorado Potato Beetle, Corn Rootworms, Cotton Boll Weevil, Cotton Fleahopper, Cotton Thrips, Crickets, Cutworms, Egyptian Alfalfa Weevil, European Chafer, Eye Gnats, False Wireworms, Flea Beetles, Garden Webworm, Grasshoppers, Japanese Beetle, Leaf Miners, Lygus Bugs, Mormon Cricket, Mosquitoes, Narcissus Bulb Fly, Onion Maggot, Onion Thrips, Rapid Plant Bug, Rice Leaf Miner, Salt Marsh Sand Fly, Seed Corn Maggot, Spittlebug, Strawberry Root Weevils, Strawberry Rootworms, Sugar Beet Root Maggot, Sweet Clover Weevil, Tarnished Plant Bug, Tuber Flea Beetle, Western Harvester Ant, White Fringed Beetles, White Grubs (June Beetles), Wireworms ...and many others.

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